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Head Office—British Museum (Natural History), Cromwell Road, London, S.W.7.

Publication Office and Library—41, Queen's Gate, London, S.W.7.

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ERRATA.

Page 151, 3 lines from end, formula (1) should read $P_r = K_A L^2 c$

Page 152, line 6, formula (3) should read $\frac{P_r}{w} = \frac{K_A L^2 c}{K_w L^3}$

Page 152, line 8, the formula should read $\frac{P_r}{w} = \frac{K_A c}{K_w \sqrt[3]{w/K_w}}$

Page 152, line 10, the formula should read

$$\log \frac{P_r}{w} = \log \frac{K_A}{K_w} + \log c - \frac{1}{3} \log w + \frac{1}{3} \log K_w =$$

$$\log c - \frac{1}{3} \log w + \text{const.}$$

Page 219, 6 lines from end, for " × " read " x "

Page 407, line 19, for " (Gerst.) " read " Gerst. "

Page 445, 6 lines from end, for " (L.) " read " L. "

Page 449, 14 lines from end, for " 90-100 per cent. " read " 75-100 per cent. "

Page 449, 8 lines from end, for " 90 per cent. " read " 97 per cent. "

Page 530, line 23, for " sodium nitroresylate " read " sodium dinitroresylate "

Page 540, line 6, for " sodium nitroresylate " read " sodium dinitroresylate "

Page 588, 9 lines from end, for " Morph. Ökol. Tiere " read " Z. Morph. Ökol. Tiere "

Page 605, last line, for " an electrometric " read " the "

Page 606, line 1, for " N/10 potassium thiocyanate " read " N/50 potassium thiocyanate "

Page 658, last line, for " isolation " read " insolation "

Page 731, 10 lines from end, for " been " read " between "

Page 745, line 31, for " (Wocke) " read " Wocke "

Page 810, last line, for " 63 " read " 69 "

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R THE BREEDING OF THE RED LOCUST IN CAPTIVITY.

By F. O. ALBRECHT, F.R.E.S.

E.M.N.

International Red Locust Control Service, Abercorn, N. Rhodesia.

Adult Locusts and Humidity.

Considerable difficulties have been experienced in the past in keeping adults of the Red Locust, *Nomadacris septemfasciata* (Serv.), alive in cages for any length of time. No satisfactory explanation as to why they die in captivity has so far been given (Hamilton, 1936).

In 1950, two thousand adult locusts collected in the Rukwa Valley, Tanganyika Territory, were kept in wire gauze cages in Abercorn and all died within ten days.

The following observations on these locusts were made :—

- (1) The relative humidity of the air often fell to 35 per cent. and below.
- (2) The locusts were noticed to drink droplets of water sprinkled in the cages and to munch damp soil.
- (3) The faeces of the caged locusts were in the form of dry pellets.
- (4) The caged locusts were restless at low humidity ; little feeding was observed and most of the locusts appeared to be searching for a way out in the upper parts of the cage.
- (5) Dying locusts were dissected ; the most striking feature appeared to be the very low water content although fat matter was abundant.
- (6) Males appeared to be less resistant than females and died sooner ; this might be explained if death were due to excessive evaporation which would be relatively greater from the males because of their smaller size.

It became evident that the caged locusts were subject to desiccation at low humidity and that they were unable to replenish the water lost by evaporation. This, however, raised the important problem of how the locusts maintain their water balance in the dry conditions of the Rukwa Valley during the dry season, and why they are not able to do so when kept in cages ; whether the water balance is maintained by moving into more humid microclimates or by excessive feeding due to increased appetite caused by flight.

The solution of this problem is not yet known but the fact that the caged locusts were subject to desiccation led to the idea of reducing evaporation by maintaining humidity in cages as near as possible to the saturation point. Glass cages were used for this purpose and these have given most satisfactory results ; considerable numbers of adults were kept in this way throughout the 1951 dry season with a mortality of less than 2 per cent. Humidity was kept high by spraying water in the cages several times a day.

Sexual Maturation.

Only one annual generation of the Red Locust occurs in the outbreak areas, the adult diapause lasting approximately eight months. The question arises as to whether the diapause can be broken and whether this long diapause is inherent in the Red Locust or is dependent on the climatic conditions in the outbreak areas ; further, whether in the absence of the dry season there is any diapause. Uvarov (1933) obtained one full generation of the Red Locust in London and reduced the adult diapause to approximately three months. Hamilton (1936) stated that two generations a year are possible provided a diapause occurs after every two such generations. Morant (1947) summarised evidence on swarm breeding of the Red Locust in Uganda suggesting more than one annual generation.

That the diapause is dependent only on climatic conditions is now quite definitely established since three successive generations of the Red Locust in 15 months have been obtained in our cages. Eggs were originally transported from the Rukwa Valley to Abercorn in December, 1950. Hatchings started on the 16th December and continued until the 9th January, the young hoppers being transferred to a glass cage of 20 cubic feet capacity. Four hundred adults were subsequently obtained, the first appearing on 26th March.

The period from the first hatching to the appearance of the first adult was 98 days. Overcrowding in the cage made it almost impossible to obtain detailed information on the duration of the hopper instars but it was approximately 19 to 21 days for the 1st instar and a minimum of 14, 12, 18, 14 and 21 days for the 2nd to the 6th instars respectively.

The relative humidity in the cage varied from 70 per cent. to 90 per cent. and the temperature as shown in Table I.

TABLE I.
Temperature conditions—first generation.

Month	Maximum day temperatures (°C.)	Mean maxima	Minimum night temperatures	Mean minima
January ...	21-32	27	13-16	15
February ...	21-41	30	13-15.50	15
March	23-49	37	13-15.50	15
April	25-50	39	11.50-15.50	14

Egg laying started on the 4th July, 1951, approximately three months after the appearance of the first adults, but bulk laying occurred in August only. A total of 285 egg pods was laid by 129 females from the 4th July to the 2nd November, representing 2.20 egg pods per female. The average number of eggs per egg pod was 64.56 and the average number of eggs laid per female was 142 ; Burnett (1951) found 182 eggs per female and Faure (1935) 200.

The second cage generation was reared in the Central Rukwa and was started on the 27th September, 1951. The temperatures varied far less than those recorded for the first generation, as will be seen from Table II; the relative humidity was 70–100 per cent.

TABLE II.
Temperature conditions—second generation.

Month	Maximum day temperatures (°C.)	Mean maxima	Minimum night temperatures	Mean minima
16th–30th Sept. ...	41–49	44	16–20	18
1st–31st Oct. ...	40–45	42	18–24	21
1st–15th Nov. ...	43–49	46	19–22	21
16th–30th Nov. ...	40–56	47	18–21	20
1st–15th Dec. ...	45–58	53	18–20	19

The minimum duration in days of the 1st to the 6th instars was 8, 6, 7, 8, 9 and 10 days respectively.

The first adult appeared on 14th November, 1951, with a minimum duration of the complete hopper life of 48 days. This figure compared favourably with the minima (45 and 49 days) recorded by Hamilton (1936).

Copulation was first observed on 9th December and the first egg pods were laid on the 14th of the same month, only 30 days after the appearance of the first adults. Laying continued until the 11th January, 1952. The average number of egg pods per female was 2.22.

Unfortunately all eggs laid in December died due to overheating; the first hatchings from eggs laid in January occurred on the 2nd February and the first adults emerged on the 13th April 1952. The minimum duration of the complete hopper life in this third generation increased to 71 days owing to the very much lower temperature.

Suggestions for Breeding in the Laboratory.

In the present experiments, it has not been possible to control climatic conditions to any great extent owing to the apparatus used. Considerable loss of time has therefore occurred. That three generations could be obtained under controlled conditions in one year, instead of 15 months is almost a certainty. No doubt sexual maturation, hopper life cycle, and egg incubation periods could be shortened by appropriate conditions of temperature and humidity.

The above information suggests that the Red Locust could be successfully bred in a laboratory with temperature and humidity control.

The following temperatures are suggested here :—

Night ...20°C.

Day ...35–45°C. for breeding of hoppers.

35–50°C. for shortening the period of sexual maturation of the adults.

A constant day temperature of 40°C. should also be tried. Relative humidity should be kept as high as possible at the above temperatures (except during the emergence and drying period of the fledglings).

The failure of field breeding experiments in the past has almost certainly been due to insufficient humidity. The literature on the subject shows that, in the laboratory, whilst sufficient humidity was applied (90 per cent. R.H., Hamilton), the temperatures were too low (100°F., Hamilton). Hoppers kept by the writer under somewhat similar conditions produced a small number of adults which never attained sexual maturity.

The minimum egg incubation period appears to be 17 days but only one of 92 overheated egg pods hatched in this time. However, eggs hatch in approximately 30 days when kept in moist sand at 30°C. and by using an incubator it should be possible to reduce this period to 25 days which is the average incubation period experienced in our cages.

The shortest duration observed for each stage of the Red Locust life cycle is given here as a guide for breeding: eggs, 25 days; hoppers, 48 days; adults to egg-laying, 30 days, giving a total of 103 days.

Red Locusts lay willingly in glass tubes filled with sand (similar in size to those used for other locust species) provided the tubes are placed against the glass wall nearest to the source of light, since egg laying normally occurs between sunrise and 10 a.m. and, in field cages, females about to lay invariably move towards the rising sun. This may be the reaction which in nature induces laying females to move out of tall grass into well lighted bare patches of soil.

Stridulation, by males only, takes place during copulation. Females stridulate during egg laying but normally only when laying is almost completed.

I am indebted to Dr. B. P. Uvarov, C.M.G., F.R.S., and Dr. D. L. Gunn, for their kind encouragement and advice.

References.

- BURNETT, G. F. (1951). Field observations on the behaviour of the Red Locust (*Nomadacris septemfasciata* Serville) in the solitary phase.—*Anti-Locust Bull.*, no. 8, 36 pp., 2 maps, 6 figs.
- FAURE, J. C. (1935). The life history of the Red Locust (*Nomadacris septemfasciata* (Serville)).—*Bull. Dep. Agric. S. Afr.*, no. 144, 32 pp., 1 map, 5 col. pls.
- HAMILTON, A. G. (1936). The relation of humidity and temperature to the development of three species of African locusts—*Locusta migratoria migratorioides* (R. & F.), *Schistocerca gregaria* (Forsk.), *Nomadacris septemfasciata* (Serv.).—*Trans. R. ent. Soc. Lond.*, **85**, pp. 1–60, 2 pls., 26 figs.
- MORANT, V. (1947). Migrations and breeding of the Red Locust (*Nomadacris septemfasciata* Serville) in Africa, 1927–1945.—*Anti-Locust Mem.*, London no. 2, 55 pp., 3 maps, 5 figs.
- UVAROV, B. P. (1933). Preliminary experiments on the annual cycle of the Red Locust (*Nomadacris septemfasciata* Serville).—*Bull. ent. Res.*, **24**, pp. 419–420.
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2 THE *ANOPHELES HYRCANUS* GROUP IN SOUTH-EAST ASIA (DIPTERA : CULICIDAE).

By J. A. REID, M.Sc.

Entomologist, Institute for Medical Research, Kuala Lumpur, Malaya. 23

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The term south-east Asia has been used here in the same sense as it was by Dobby (1950) ; this has the advantages of convenience and familiarity, and is not yet too closely defined. Roughly it is the Oriental region less India and China. The forms of *Anopheles hyrcanus* (Pall.) found in those two countries have also been considered, but this paper is chiefly concerned with the *hyrcanus* of south-east Asia, particularly the Malay Peninsula.

A. hyrcanus is one of the most widespread and common "species" of *Anopheles*, and certainly one of the most variable. Though readily distinguished from other south-east Asian species, it is very similar to its close relative and counterpart in Africa, *A. coustani* Lav. which is also very variable.

A. hyrcanus has an enormous range, from Spain and southern France in the west to Pacific Russia in the east, and the Oriental region in the south. Owing to its variability a number of different forms have been described, but for the most part it has not been possible in the past to distinguish these from one another with certainty. The descriptions have mostly been of forms common in a particular region, such as *sinensis* Wied. in China, *nigerrimus* Giles in India, and *mesopotamiae* Chr. & Kh. Chand in Mesopotamia, but where such forms occur together the descriptions have not afforded any critical means of separating them. Edwards (1932) and Christophers (1933), in their splendid work on the systematics of the Anophelines, realised this and treated most of the names as synonyms or at most as varieties of *hyrcanus* Pallas, 1771, whilst recognising that at some future time further study might require some of the names to be re-instated. That time has now come for *hyrcanus*, and for other species of *Anopheles* such as *leucosphyrus* Dön. and *punctulatus* Dön., in which recent studies have shown that, as with *A. maculipennis* Mg., we are dealing not with one variable species, but with a group of closely similar species widely overlapping in their ranges, not interbreeding and some at least polytypic, *i.e.*, having different geographical forms.

The recognition and satisfactory definition of these closely allied or sibling species must be done on the spot where there is unlimited material available, and all stages of the life history can be studied alive or dead, and biological as well as morphological differences can be noted. When the species have been disentangled

in this way and can be recognised with certainty, it is then time to study museum collections, which are indispensable for settling questions of nomenclature by reference to types, and for gaining some knowledge of the ranges of the species and their geographical variation. The last two points are of some importance, because describing sibling species of *Anopheles* from say Malaya alone, without reference to specimens from the surrounding countries, is a practice to be avoided if possible as it may only increase confusion. The smallness of the differences and the modifying effects of geographical variation upon these, may make it difficult for workers in surrounding countries to decide whether they have the same species or not, if these are described only from a small part of their range. Although the formal descriptions which follow are in fact based entirely on Malayan specimens, notes are given on geographical variation when this has been seen, and the range of each species is recorded so far as it is known from examination of specimens in various collections.

The *Anopheles* (*Anopheles*) *hyrcanus* group belongs to the series *Myzorhynchus*, and is distinguished from most other species by the presence in the female of a tuft of dark scales on the clypeus on each side; the clypeus is usually bare in *Anopheles*. This character, and the more readily observed pale bands on the palps and ventral tuft of scales on the seventh abdominal segment, normally serve to identify the species (females). However, there are few related species which also have clypeal scales, and may in addition have banded palps and scales on the seventh sternite. The closely allied *coustani* group, which is the African counterpart of *hyrcanus*, has these characters (except that the scales of the seventh sternite are sometimes absent), but the fifth hind tarsal segment is all white. Three other African species which have clypeal scales are *paludis* Theo. (with 5th hind tarsal segment white as in *coustani*), *fuscicolor* van Som. (with tip of female palps dark, and a secondary or ventral cleft in the pupal trumpet), and *symesi* Edw. (with stem of vein 5 all pale and no scales on the seventh sternite). The only other African *Myzorhynchus*, *A. obscurus* Grünb., lacks clypeal scales and differs markedly from *hyrcanus* in other respects. One Japanese species, *A. sineroides* Yam., has clypeal scale tufts, banded palps and scales on sternite seven, but the costa has two additional pale marks towards the base rarely present in *hyrcanus*, the outer clypeal hairs of the larva have much fewer branches (8-25) than *hyrcanus* (30-90), and the phallosome leaflets are not serrated and are of equal length. The related *A. koreicus* Yam. & Watan. has no clypeal scales and differs markedly from *hyrcanus* in other respects. Among common Oriental species, *A. barbirostris* is probably the closest ally of *hyrcanus*, but it lacks the clypeal scales, has entirely dark palps, a secondary cleft in the pupal trumpet and several other considerable differences.

In these remarks on related species I have been greatly helped by Dr. Alan Stone of the U.S. National Museum who kindly examined specimens of *sineroides* and *koreicus* for the clypeal scales, and by Mrs. E. C. C. van Someren of the Medical Research Institute, Nairobi, and Mr. P. F. Mattingly of the British Museum (Nat. Hist.) who helped with the African species. Mrs. van Someren kindly gave me specimens of *A. coustani*. The literature consulted was Christophers (1933, p.13), Evans (1938), van Someren (1947), and W. J. La Casse and S. Yamaguti (Mosquito fauna of Japan and Korea, 213 pp., U.S. Army, 1950).

From the foregoing it is evident that a more complete definition of *hyrcanus* would be as follows: female with a tuft of scales on the clypeus on each side, palps with pale bands (usually four) one of which is always apical, seventh sternite with a tuft of scales, fifth hind tarsus not all white (rare exceptions occur in *argyropus*), stem of vein five always with a well defined dark mark towards the base, male phallosome leaflets with some serrations and of unequal length, pupal trumpet without a secondary cleft, outer clypeal hairs of larva with 30-90 branches.

In the *A. umbrosus* group, *separatus* bears a superficial resemblance to *hyrcanus* and has pale bands on the palps, but lacks the clypeal and seventh abdominal scale

tufts ; also the wing is different as it conforms to the *umbrosus* type, lacking pale scales at the base of veins 1 and 5. This difference in the wings is useful in distinguishing the males of *hyrcanus* and *separatus* which are otherwise very similar, though the ornamentation of the palps is somewhat different, and male *separatus* commonly have a considerable number of pale scales on the apparent dorsal surface of the eighth abdominal segment, these being few or absent in *hyrcanus*.

This study of *Anopheles hyrcanus* was commenced in 1940 when Hodgkin, then the Entomologist of this Institute, drew my attention to Crawford's work (1938) on the pupae of Malayan *Anopheles* including *hyrcanus*, and the paper by Venhuis (1939) describing *A. hyrcanus* variety X, a malaria vector in Java and Celebes. Hodgkin himself had earlier shown (1933) that *hyrcanus* could be a vector of malaria in Malaya, and the species had long been regarded as of some importance in the transmission of malaria and filariasis. I therefore took up the work with enthusiasm, and being then a research student, was able to devote to it the large amount of time that was required. It would not be possible now to undertake another study on quite this scale ; in particular the labour of counting such large numbers of larval hairs (Table VI) is now beyond my resources of time and patience.

Keys to Malayan Forms.

Additional characters are given in the section on identification under each species, and remarks on forms not occurring in the Malay Peninsula will be found at appropriate places. Many of the characters used in the keys are illustrated by figures in the text. The terminology of Christophers (1933) has been used.

ADULTS.

1. Pale bands on hind tarsi narrow, fourth segment without basal pale band.....2
Pale bands on hind tarsi moderately broad to very broad, fourth segment with basal pale band.....4
2. With very short apical fringe spot, between veins 1 and 2.2. Basal dark mark on 5 fairly long, approaching to within its own length or less of the upper mark on 6. Coxites of the male genitalia without pale scales on the apparent dorsal surface.....**lesteri* Baisas & Hu
Apical fringe spot not very short, extending at least from 2.1 to 3. Basal dark mark on 5 short, separated by its own length or more from the upper mark on 6. Coxites of the male genitalia with pale scales.....3
3. Wing pattern sharp, the dark marks short and well defined. Tip of vein 1 pale, apical fringe spot rather short commencing at 2.1, no fringe spot at 5.2, apical dark mark on 6 not longer than that on 5.2, no pale scales on 1 between subcostal and preapical pale spots.....*crawfordi*, **sp. n.**
Wing pattern blurred. Tip of vein 1 dark, apical fringe spot longer commencing at or above vein 1, fringe spot usually present at 5.2, apical dark mark on 6 longer than that on 5.2, some pale scales on 1 between subcostal and preapical pale spots.....*sinensis* Wied.
4. Wing pattern bright, the dark marks mostly short and well defined. Basal half of the costa always with some pale scales, basal dark mark on 5 separated by its own length or more from the upper mark on 6. Seldom more than four propleural setae.....*indiensis* Theo.
Wing pattern darker, more or less blurred. Basal dark mark on 5 approaching to within its own length or less of the upper mark on 6. Basal half of the

* The typical Philippine form does not have a very short apical fringe spot. Species D2, especially females, would run down here, but could be separated as indicated on page 44,

- costa without pale scales, except *nigerrimus* which seldom has less than seven propleural setae.....5
5. Third hind tarsal pale band seldom longer than the fifth segment. Costa usually with one or two pale scales towards the base, often a fringe spot at 5.2, tip of the ♀ abdomen on each side (eighth tergite) usually with a few narrow scales. Male palps with a pale band on the base of the third segment...*nigerrimus* Giles
Third hind tarsal pale band longer than the fifth segment. Basal half of costa without pale scales, no fringe spot at 5.2. Seldom any scales at the tip of the abdomen. Male palps without pale band on the base of the third segment.....6
6. Hind tarsal pale bands very broad, third band more than three-quarters the length of the fourth segment; mid-tarsal bands narrow, the third band about one-quarter the length of the third segment. Wing dark, no pale scales on vein 1 between subcostal and preapical pale spots.....*argyropus* Swellengr.
Hind tarsal pale bands not so broad, third band usually less than three-quarters as long as the fourth segment; mid-tarsal bands broad, the third band one-third or more as long as the segment. Wing lighter, with pale scales, usually numerous, on vein 1 between subcostal and preapical pale spots.....*peditaeniatus* Leic.

FOURTH-STAGE LARVAE.

This key has been constructed for the identification of living larvae, rather than prepared specimens. Separation of the larvae is not easy, but most of the characters are illustrated by figures in the text.

1. Mesothoracic hair 5 small, with sinuate horizontally spreading branches arising together from the base.....*peditaeniatus* Leic.
Mesothoracic hair 5 not so, the branches straight, stiff and more or less erect.....2
2. Sutural hair with numerous branches (13—23), commonly 17. Antennal shaft rather slender, usually with rather large, coarse, erect teeth. Tergal plate on abdomen VIII between two-thirds and three-quarters as long as wide, usually tapering posteriorly more or less in the form of a truncated wedge. Pigmentation of palmate hairs usually uniform and rather dense. Saddle hair strong, at least as long as the width of segment VIII. Usually seven long teeth on the pecten, rarely fewer.....*argyropus* Swellengr.
Without this combination of characters. If the sutural hair has more than 12 branches, then either tergal plate VIII is large and transversely rectangular, less than two-thirds as long as wide, and the palmate hairs are large with the pigmentation generally less dense and not uniform, paler towards the base of the leaflets (*sinensis*, *nigerrimus*), or the saddle hair is weak, less than the width of segment VIII, and the pecten rarely has more than six long teeth (*indiensis*)3
3. Palmate hairs large, the pigmentation often not uniform nor very dense, but paler basally, often extending well into the tips of the leaflets. Tergal plate VIII transverse, usually less than two-thirds as long as wide. Spiracles large.....4
Palmate hairs somewhat smaller, pigmentation commonly uniform and dense and not extending much into the tips of the leaflets. Tergal plate VIII usually less transverse, two-thirds or more as long as wide. Spiracles smaller.....5
4. Abdomen VI, hairs 5 and 9 with 6—11 branches, average eight. Sutural hair with 8—13 branches, average 11.....*sinensis* Wied.
Abdomen VI, hairs 5 and 9 with 2—5 branches, usually three or four. Sutural hair with 12—24 branches, average 17.....*nigerrimus* Giles

5. Sutural hair with 11–17 branches, average 13. Abdomen III, hair 9, 10–16 branches. Saddle hair weak, not as long as the width of segment VIII. Pecten seldom with more than six long teeth.....*indiensis* Theo.
Sutural hair with 5–11 branches, average nine. Abdomen III, hair 9, 5–11 branches. Pecten seldom with less than seven large teeth. Saddle hair strong or weak.....6
6. Segment II, hair 5 with 6–10 branches, average nine. Saddle hair strong, about as long as the width of segment VIII.....*lesteri* Baisas & Hu
Segment II, hair 5 with 10–18 branches, average 13. Saddle hair weak, usually shorter than the width of segment VIII.....*crawfordi*, **sp. n.**

PUPAE.

1. Branches on spine VIII absent or much reduced. Paddle with border teeth extending beyond three-quarters of its length.....2
Spine VIII with well-developed branches. Border teeth of paddle seldom extending as much as three-quarters of its length.....3
2. Sides of trumpet with parallel vertical wrinkles. Hair 2* on V with many (17–40) branches.....*argyropus* Swellengr.
Sides of trumpet not so. Hair 2 on V with few (1–6) branches.....*peditaeniatus* Leic.
3. Rim of trumpet with thickened and coarsely toothed areas.....4
Rim of trumpet thin, uniform.....5
4. Hair 2 on abdomen VI with 2–4 branches, 5* on V with many (30–45) branches.....*crawfordi*, **sp. n.**
Hair 2 on VI with 5–10 branches, 5 on V with 12–20 branches.....*lesteri* Baisas & Hu
5. Hair 5 on V with rather few (9–24) branches. Wing case with rows of round dark spots. Tip of antennal case usually light.....*sinensis* Wied.
Hair 5 on V with many (30–60) branches forming a tuft. Wing case without round dark spots, though there may be a vague pattern of veins and crossbars. Antennal case often dark at the tip.....6
6. Usually lightly pigmented with contrasting sharply-defined dark tip to the antennal case. Base of paddle light.....*indiensis* Theo.
Pigmentation usually moderate to dark with the paddle base more or less dark. Tip of antennal case light, or with less sharply defined dark pigmentation.....*nigerrimus* Giles

EGGS.

1. Deck divided into two areas at each end of the egg, about one-sixth its width.....*indiensis* Theo.
Deck complete2
2. Upper surface of egg concave in side view. Ends more or less rounded viewed from above.....3
Upper surface of egg flat, or slightly convex in side view. Ends with more or less projecting points viewed from above.....4

* Nomenclature of Knight and Chamberlain (1948), see p. 17.

3. Deck wide, about one-third the width of the egg.....*sinensis* Wied.
Deck much narrower, about one-seventh the width of the egg.....*nigerrimus* Giles
4. Deck roughly one-sixth the width of the egg.....*peditaeniatus* Leic.
Deck very narrow, less than one-tenth (*lesteri*), or less than one-twentieth as wide
as the egg.....(*argyropus* Swellengr., *crawfordi*, **sp. n.**)

The full details of larval chaetotaxy and other measurements are to be found in Tables IV-XIII.

Anopheles (Anopheles) sinensis Wied. 1828.

A. plumiger Dönitz, 1901, Hong Kong ; type in Zool. Museum, Berlin (Yamada p. 230).

A. jesoensis Tsuzuki, 1901, Hokkaido, type unknown (Yamada, p. 223).

Pupal type A of Crawford, 1938.

Adult Female.

Head.—Proboscis dark, except that there may be signs of a very few small pale scales just before the labella (11/20), somewhat shaggy towards the base, mean length in five specimens 2.08 mm., slightly longer than the fore femur, ratio fore femur/proboscis=0.86. Palps shaggy, especially basally, shorter than the proboscis by about the length of the labella ; dark, with four pale bands of which the fourth or apical and the third are the best developed, occasionally (5/50) these two coalesce. The first and second (basal) pale bands are sometimes very weak or absent (8/50). Near the base of the palps medially, basal to the first pale band, there are usually (42/50) some pale scales, sometimes numerous enough to form a definite pale area, and there may be (20/50) a few pale or steely grey scales scattered between the pale bands. Clypeus dark with a tuft of closely set dark scales on either side which curve forwards and downwards. Antennae with a few pale scales on the rim of the torus posteriorly, and a variable number of pale scales on about the first six flagellar segments ; most numerous on the first segment where a few ventrally may be dark. Vertex narrow with white setae and scales, a median patch of pale scales on the occiput which may be continued downwards on either side as a narrow area of slightly pale scales bordering the eye ; remaining head scales dark.

Thorax.—Mesonotum viewed from above and behind, greyish pruinose interrupted by dark longitudinal bands and marks ; one narrow median dark band runs from the anterior border of the mesonotum to the scutellum where it is expanded. On either side of this median band anteriorly is a broader curved band lying parallel to the anterolateral area or fossa which is partly dark. Posterior to each fossa is an oblong dark eye spot, and behind this reaching to the scutellum there may be a faint ridge or band slightly darker than the adjacent area. Scales on the anterior promontary of the mesonotum, pale medianly to yellowish laterally with a few dark ones ventrolaterally. Anterior pronotal lobes with a tuft of dark scales. Setae of the mesonotum sparse, decumbent, mainly pale golden. Scutellum greyish pruinose. Halteres with dark apical scales. Pleurae dark testaceous, slightly pruinose, without scales except an occasional one associated with one or other of the groups of setae, usually the propleural or sternopleural. Propleural setae 4-10 average 6.9. Details of the other pleural setae and of the upper mid-coxal setae will be found in Table VI in the Appendix.

Legs (fig. 3 a).—Dark brown above, paler beneath. Coxae, particularly the mid ones, with some groups of pale scales among the setae. Trochanters with dark scales, extreme base of the fore femur and outer face of fore trochanter with pale scales. Femora apically darker to black, usually with a pale fleck at the tibial joint, especially on the mid and hind legs. Tibiae of mid and hind legs with a pale fleck

or band at base and apex. Tarsal segments I to III always with apical pale bands. Fore tarsi ; second band the longest varying from 2.6–4.7 times as long as wide, usually about three times which corresponds to about one-quarter the length of the segment. No basal bands, *i.e.*, the apical pale bands do not extend on to the bases of the next segments. Very occasionally (3/50) a trace of a band on the apex of tarsus 4. Mid tarsi ; pale bands similar to those of the fore tarsi but shorter, second band from 1.3–2.9 times as long as wide, average 1.7. Hind tarsi ; bands 1, 2 and 3 of nearly equal length, sometimes 2 and 3 slightly longer, 3 varying from 0.8 to 2.6 times as long as wide, average 1–1.5. Bands 1, 2 and 3 are apical only, very rarely 3 extends slightly on to the base of segment IV which usually has a small apical band that very occasionally extends slightly on to the base of the fifth segment.

Wings (fig. 1 *a*).—Length from root to apex less the fringe 3.00–4.33, average 3.84 mm. Costa dark from base to subcostal pale area and between this and the pre-

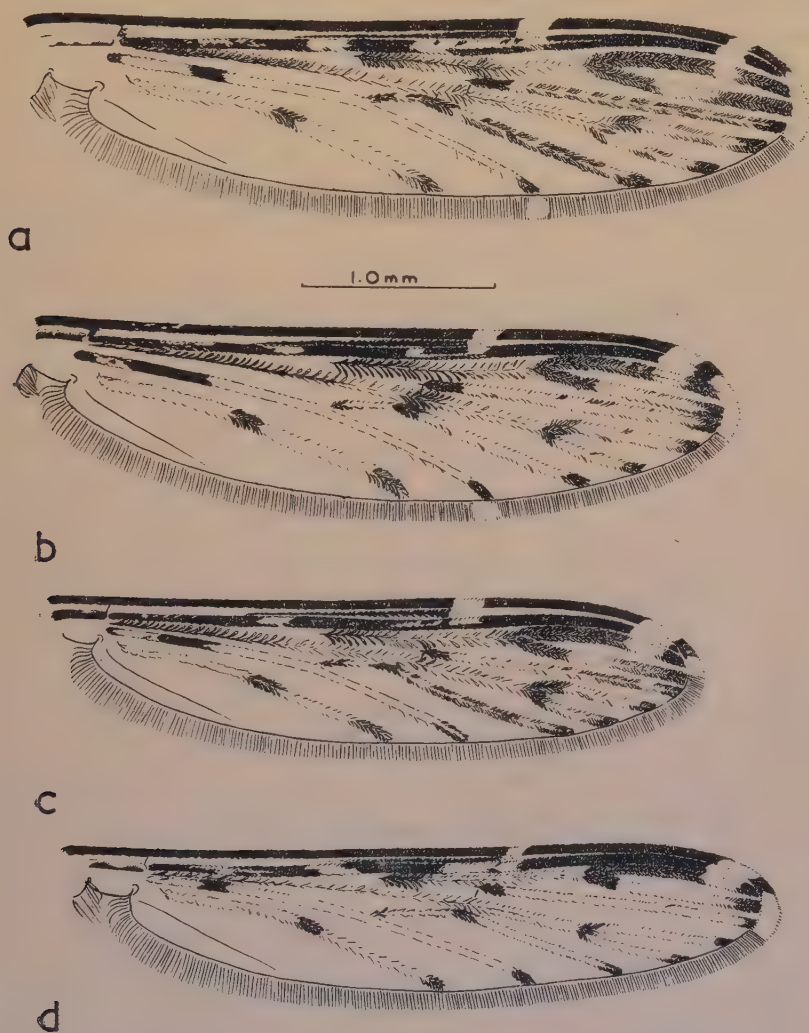


Fig. 1.—Wings : (a) *sinensis*, (b) *nigerrimus*, (c) *lesteri*, (d) *crawfordi*.

apical pale area, though occasionally (9/50) there may be one or two minute ochreous scales at, or proximal to, the humeral cross vein ; the latter bears a few scales (48/50), usually dark (42/50). Remigium with light and dark scales, those of the anterior border usually mainly light (44/50). Subcostal pale area well developed varying little in width, usually complete on vein 1 (44/50), if incomplete, there are a few dark scales on the posterior border of 1. Subcosta mainly dark, except for the tip, becoming paler basally. Vein 1 between remigium and sector pale area with scattered pale scales mostly in the middle 2/3 of this section, varying from a few (3-10) up to about half the scales pale ; sector pale area well developed, may be longer or shorter than the subcostal pale area ; middle dark mark (section between sector and sub-costal pale areas) with the distal half usually pale or mostly pale and continuous with the subcostal pale area ; always with some pale scales, but usually also some dark scales remain on the posterior border of the vein ; preapical dark mark with scattered pale scales varying from none (1/50) to about 1/3 of the scales pale ; extreme tip of vein 1 dark. Preapical pale area usually well developed on the costa and veins 1 and 2.1, occasionally (6/50) small or absent on the costa and then absent or incomplete on 2.1 (12/50), always complete on vein 1. Pale apical fringe spot usually extending from termination of vein 1 (37/50) to a little beyond 3 (48/50), sometimes a little longer than this, commencing above 1 (12/50), rarely shorter. Vein 2 with a dark mark at the base, usually pale towards the fork ; 2.1 dark from fork to preapical pale area, then dark at the tip ; 2.2 with a shorter basal dark mark followed by a pale area which gives place slowly or abruptly to dark scales towards the tip. Vein 3 with a dark base and shorter dark tip, mainly pale between but with a variable number of dark lateral scales. Vein 4 dark basally becoming

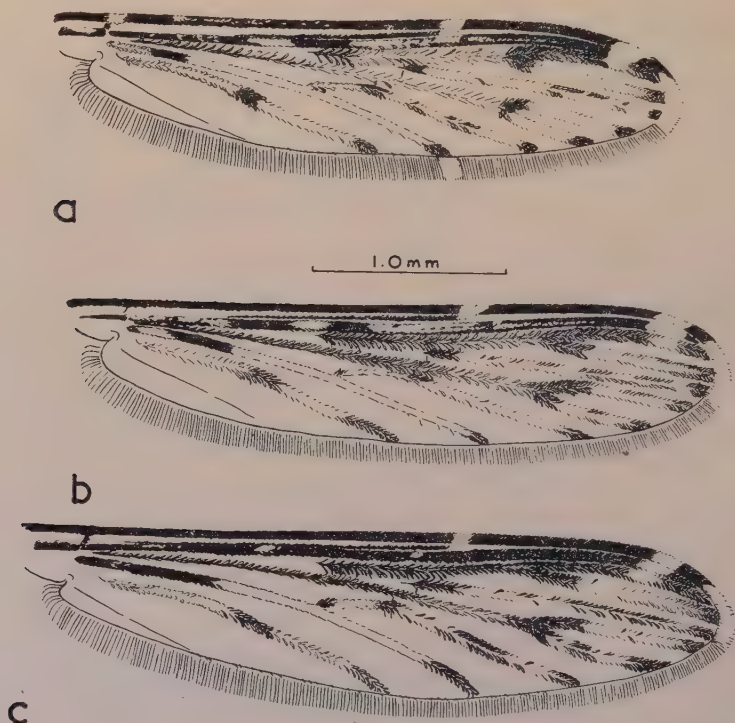


Fig. 2.—Wings : (a) *indiensis*, (b) *peditaeniatus*, (c) *argyropus*.

lighter towards cross vein r-m, between this and the fork mainly pale, a dark mark at the fork extending a little along 4.1, tips of 4.1 and 4.2 dark, mainly pale between tips and fork with a variable number of dark lateral scales. Vein 5 with one or two dark scales at extreme base, occasionally forming a definite small dark mark, after this a pale area usually (45/50) longer than or equal to succeeding (basal) dark mark, sometimes there are scattered dark scales in this pale area. From the basal dark mark to the dark mark at the tip of 5.2, entirely pale. The basal dark mark on 5, 0.2-0.4 mm. long, usually separated by at least its own length (up to 3x) from the upper dark mark on 6 (47/50). Vein 5.1 with three dark marks, one at the fork, one distal to this at the bend, and one apical, the first two sometimes fuse (9/50). Between the second and the apical dark marks the vein is pale dorsally but usually with numerous dark lateral scales, range about 8-50. Vein 6 with two dark marks, an upper about midway along its length and an apical, the upper mark shorter than the apical (49/50), the apical always longer than the apical mark on 5.2; occasionally a few dark scales basally above the upper dark mark (8/50). Fringe scales dark between termination of apical pale fringe spot and anal angle, except for a pale spot at 5.2 in 74 per cent. (37/50). Border scales pale with a few scattered dark ones.

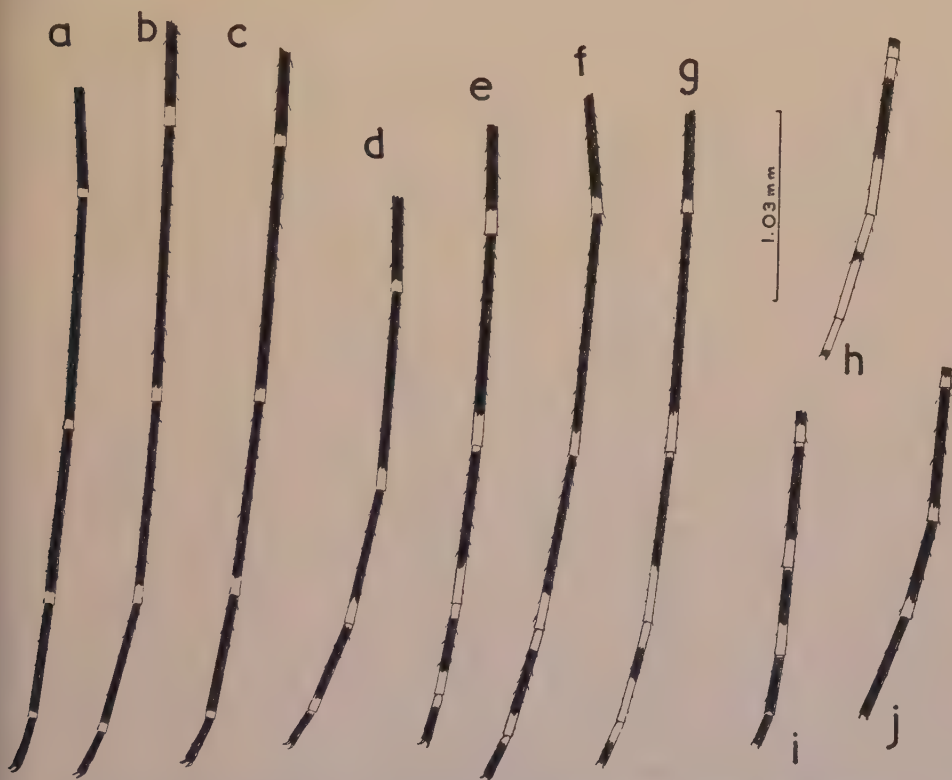


Fig. 3.—Tarsi: hind tarsi; (a) *sinensis*, (b) *crawfordi*, (c) *lesteri*, (d) *nigerrimus*, (e) *indiensis*, (f) *peditaeniatus*, (g) *argyropus*, (h) *argyropus*, a very broad banded specimen: mid tarsi; (i) *peditaeniatus*, (j) *argyropus*.

Abdomen.—Dark brown, not pruinose, with pale golden hairs. Posterior border of sternite VII with a median tuft of dark scales. No other scales except very rarely (1/25) one or two slender ones amongst the setae at the tip of the abdomen on each side (postero-lateral angles of tergite VIII).

Adult Male.—Essentially similar to the female in wing markings, tarsal banding, thoracic chaetotaxy, etc.

Head (fig. 4 *b*).—No distinct tufts of scales on the clypeus like those of the female ; any clypeal scales there may be are indistinguishable from those of the base of the palps, and *sinensis* does not seem to differ in this respect from *A. separatus* (see Reid & Hodgkin, 1950, p. 306). Basal third of the palps (2nd segment) shaggy proximally, and usually with median dorsal pale scales corresponding to the median basal pale patch on the female palps ; these pale scales may extend to the bare joint between the 2nd and 3rd segments. The base of the 3rd segment is dark and then there may be another streak of median dorsal pale scales extending to the base of the club, ornamentation of the club as in fig. 4 *b*. First flagellar segment of the antennae with one or two pale scales.

Abdomen.—Sternite VIII (dorsal after inversion) occasionally (2/10) bears one or two slender pale scales posteriorly. The apparent dorsal surface of the coxites always with pale scales, lateral scales dark. *Terminalia* (figs. 5 *a-d*, 6 *a*).—In general pattern the male terminalia of the *hyrcanus* group are very similar to those of the *umbrosus* group ; the 9th tergite bears well-developed processes, the outer parabasal spine is fairly straight, and the inner shorter, hooked and spatulate terminally. The spines of the dorsal lobe of the harpago are fused into a club and the ventral lobe bears a few large setae, of which the largest is somewhat flattened in the distal third before the tip. The phallosome bears leaflets. The terminalia seem to differ from those of the *umbrosus* group in that the outer parabasal spine is not so straight, and the club on the harpago usually shows one or two slender spines free at the tip and projecting inwards below the top of the club ; the phallosome leaflets, at least the larger ones, bear some serrations, whilst those of the *umbrosus* group are more often plain.



Fig. 4.—Male palps : (a) *nigerrimus*, (b) *sinensis*.

A. sinensis has 3–6 pairs of leaflets on the phallosome, usually 4 or 5, the leaflets of the largest pair bear teeth, usually prominent, and those of the second largest pair are usually serrate also ; there are pronounced basal teeth on one or more of the leaflets. The free spines in the club on the dorsal lobe of the harpago are not well developed. Ventral lobe of the harpago commonly with two setae, occasionally 3 or 4, one much the longest and somewhat flattened distally.

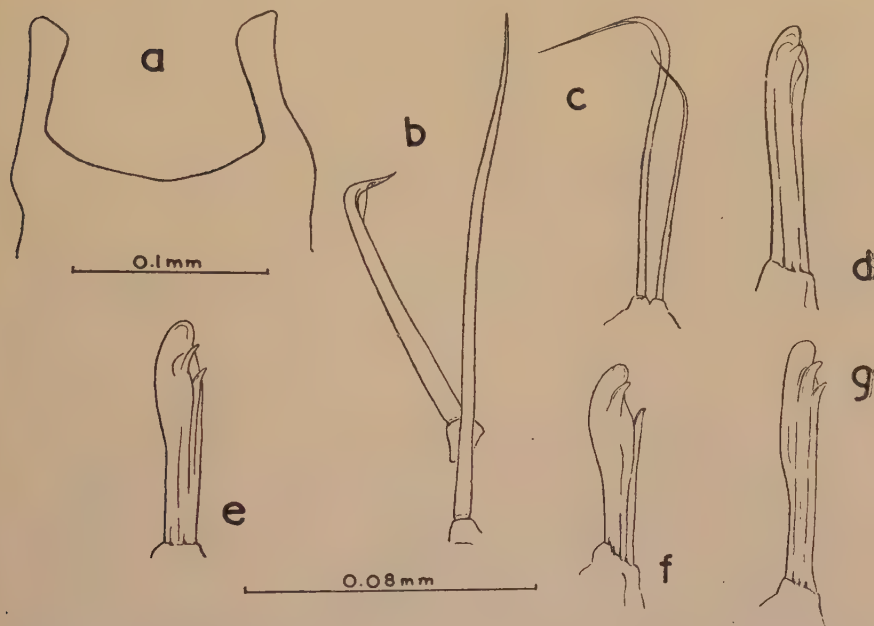


Fig. 5.—Male terminalia (these figures and the descriptions in the text are based on four specimens of each species (*crawfordi*, three)) *sinensis*; (a) processes of 9th tergite; (b) parabasal spines; (c) setae on the ventral lobe of the harpago; (d) club on the dorsal lobe of the harpago; (e) ditto *indiensis*; (f) ditto *argyropus*; (g) ditto *crawfordi*.

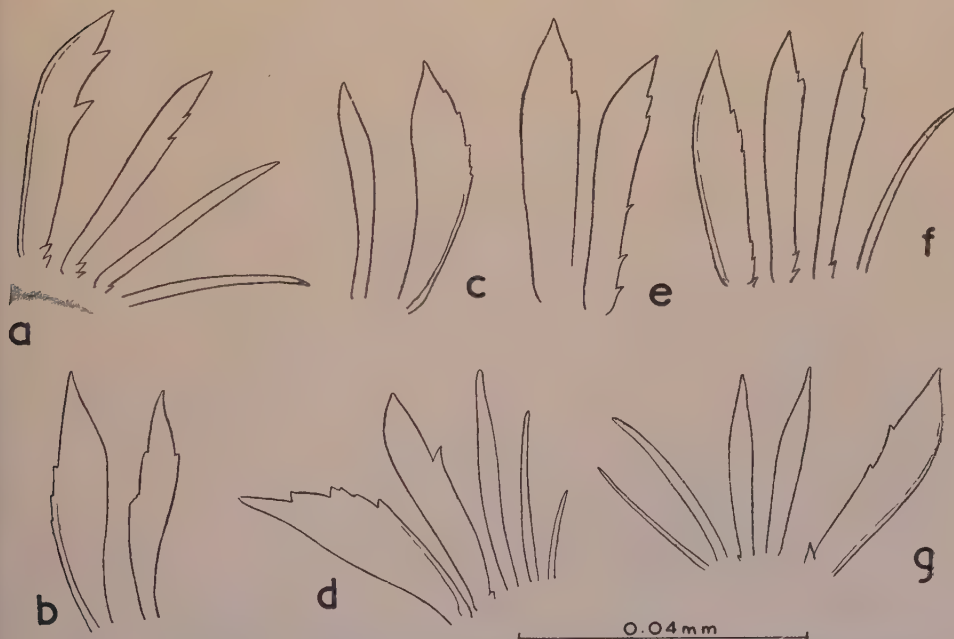


Fig. 6.—Male terminalia, phallosome leaflets (these figures and the descriptions in the text are based on four specimens of each species (*crawfordi*, three): (a) *sinensis*, (b) *nigerrimus*, (c) *indiensis*, (d) *peditaeniatus*, (e) *argyropus*, (f) *lestieri*, (g) *crawfordi*.

Larva.—When viewed by naked eye or with a hand lens the living larva is usually bright green, or occasionally yellowish green or brown; usually without any pale bands on the abdomen, or only with faint ones. Lamborn (1922a, p. 403) found pale bands on the thorax and abdominal segments III, V and VIII in *sinensis* larvae in China.

Head (fig. 8 a).—Inner anterior clypeal hairs usually simple, but in about one-third of the specimens one or both hairs may have from 2 to 4 branches. Outer anterior clypeal hairs with about 60–80 rather long slender branches, not very stiff. Sutural hair with 8–13 fairly slender branches, average 11. Antennal shaft hair of variable length, sometimes reaching beyond the end of the shaft, sometimes not.

Thorax.—Inner shoulder hair simple, or more often with 2–5 small branches towards the tip. Mesothoracic hair 5 with 4–8 erect straight branches (fig. 9 c).

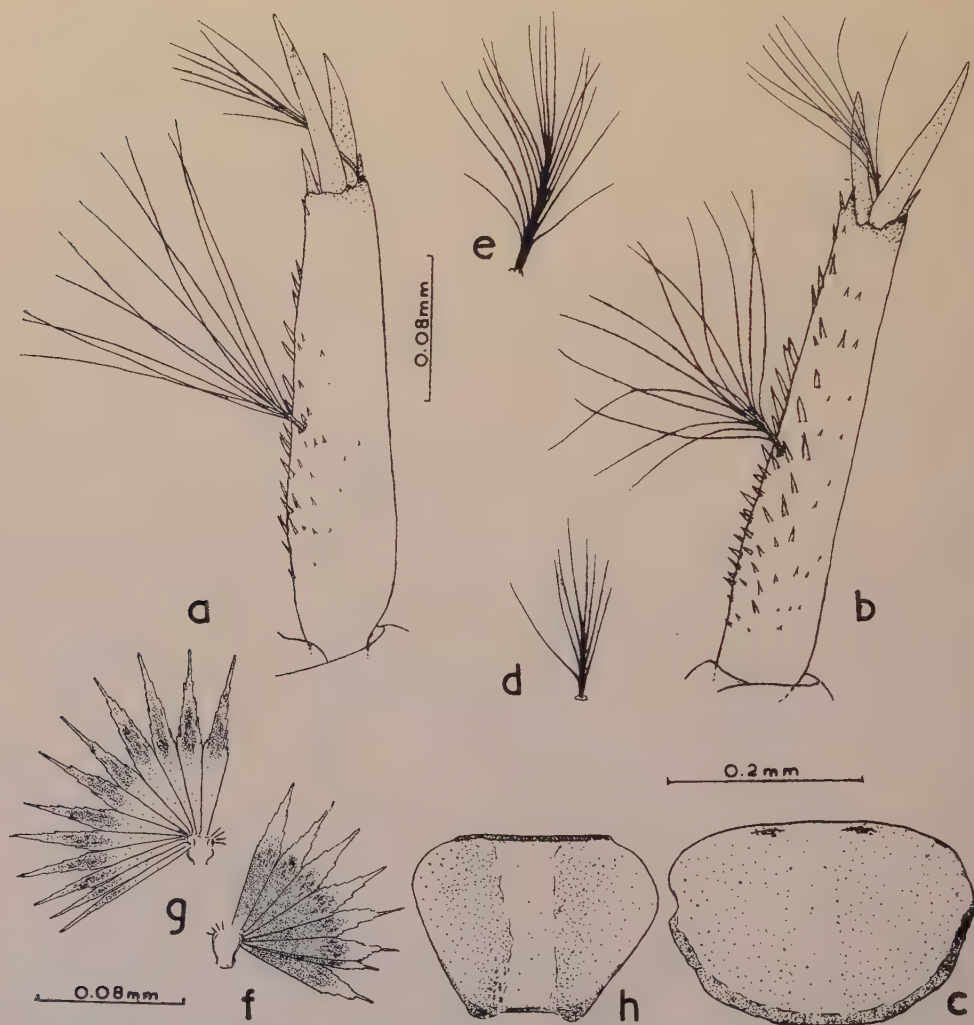


Fig. 7.—Larval structures: *nigerrimus*; (a) antenna; (c) 8th tergite plate; (e) sutural hair: *argyropus*; (b) antenna; (h) 8th tergite plate: *lesteri*; (d) sutural hair; (f) abdominal palmate hair: *sinensis*; (g) abdominal palmate hair.

Abdomen.—Palmate hairs (fig. 7 g) fully developed and pigmented on segments III–VII, large, with slender tapering points, the pigment frequently neither uniform nor very dense, but paler towards the base of the leaflets and extending into the tips. Segment V, hair 5, 4–10 branches. Segment VI, hair 5, 6–11 branches, hair 9, 6–10 (fig. 9 e). Tergal plate on segment VIII rather large, transversely rectangular, usually less than two-thirds as long as wide; ratio length/width 0.54–0.69, average 0.61. Pecten with 7–9 long teeth, commonly 8. Spiracles large (fig. 8 f). Anal gills usually longer than the saddle or segment VIII (probably of smaller size in specimens from coastal areas). Saddle hair as long as or longer than the saddle, or the width of segment VIII.

Pupa.—Crawford (1938) described and illustrated the pupae of five of the seven species described here, calling them varieties A, B, C, D and E. The brief descriptions given here are based partly on his work, but the setal nomenclature is that proposed by Knight and Chamberlain (1948).

The pupa of *sinensis* is usually very lightly pigmented but has a distinctive pattern of round dark spots arranged in lines on the wing case (fig. 11 a). These spots correspond to the cross bars between the veins in the pigmentation pattern on the wing cases of the other species. The pigmentation patterns on the pupae are not entirely reliable, but are constant enough to be helpful in identification. In *sinensis*, the tips of the antennal cases and the bases of the paddles are usually light. The trumpet (fig. 12 b, d) is large, boat shaped and bivalved without the tragus present in the *umbrosus* group (Reid & Hodgkin, 1950), and with a thin evenly crenate rim. Abdominal hairs 2 (=C) and 5 (=B) have comparatively few branches; on segment V, hair 2 has 3–13 branches, commonly 5–7, and hair 5, 9–24, commonly about 14 branches. The cuticle bears small denticles which make the lateral borders of the segments appear finely serrate. Spine VIII with 10–22 well-developed branches. Refractile border of paddle about two-thirds the length of the paddle (fig. 12 a). This is the vertical length of the refractile or toothed border compared to the vertical length of the paddle measured from root to apex, not the length measured along the curve of the border as was done by Crawford.

Egg (fig. 13 a, e).—Characterised by a wavy margined broad deck, which is about one-third the width of the egg and concave in side view. The floats have about 30–35 ribs. The lower surface, and the upper surface between the floats and the deck, are marked by a polygonal meshwork pattern.

For other illustrations of this species see: male terminalia, Christophers, 1933, ? fig. 24/7; Russell and Baisas, 1936, pl. 8, ? specimen from Hong Kong; pupa, Crawford, 1938, fig. 10; egg, Lamborn, 1922a, fig. 1a, though rather crude, this drawing and the measurements given (deck 0.06 mm. wide) are clearly of *sinensis*; Walch and Walch-Sorgdrager, 1935, pl. III, fig. 35; Baisas and Hu, 1936, pl. 2. The illustrations of larval and pupal characters of *sinensis* from Shanghai by Baisas and Hu are probably of the species as defined here which lays wide-decked eggs, but since there appear to be at least two forms in China included in *sinensis*, this is not certain.

Identification.—The adult female in Malaya is characterised chiefly by the narrow apical pale bands on the hind tarsi not extending across the joints, and the short dark mark at the base of vein 5, in combination with the blurred type of pattern in the distal field of the wing, with a full-sized apical fringe spot, pale scales on vein 1 between subcostal and preapical pale spots, and usually a fringe spot at 5.2. Other points not mentioned in the key are the absence of pale scales on the costa basally, presence of dark scales on the humeral cross vein and pale scales on the mid coxa, and propleural setae numerous, 4–10, usually 6 or more. Identification of larvae is not easy until one is familiar with the various characters and small differences. Points which may be of value and are not included in the key, are the large size and

green colour common in *sinensis*, the outer clypeal hairs usually with rather long fine branches compared to the shorter stiffer ones of most of the other species including *nigerrimus*, the inner clypeals sometimes branched distally, the lateral hair (No. 6) on abdomen III usually with more than 20 (average 23·8) branches.

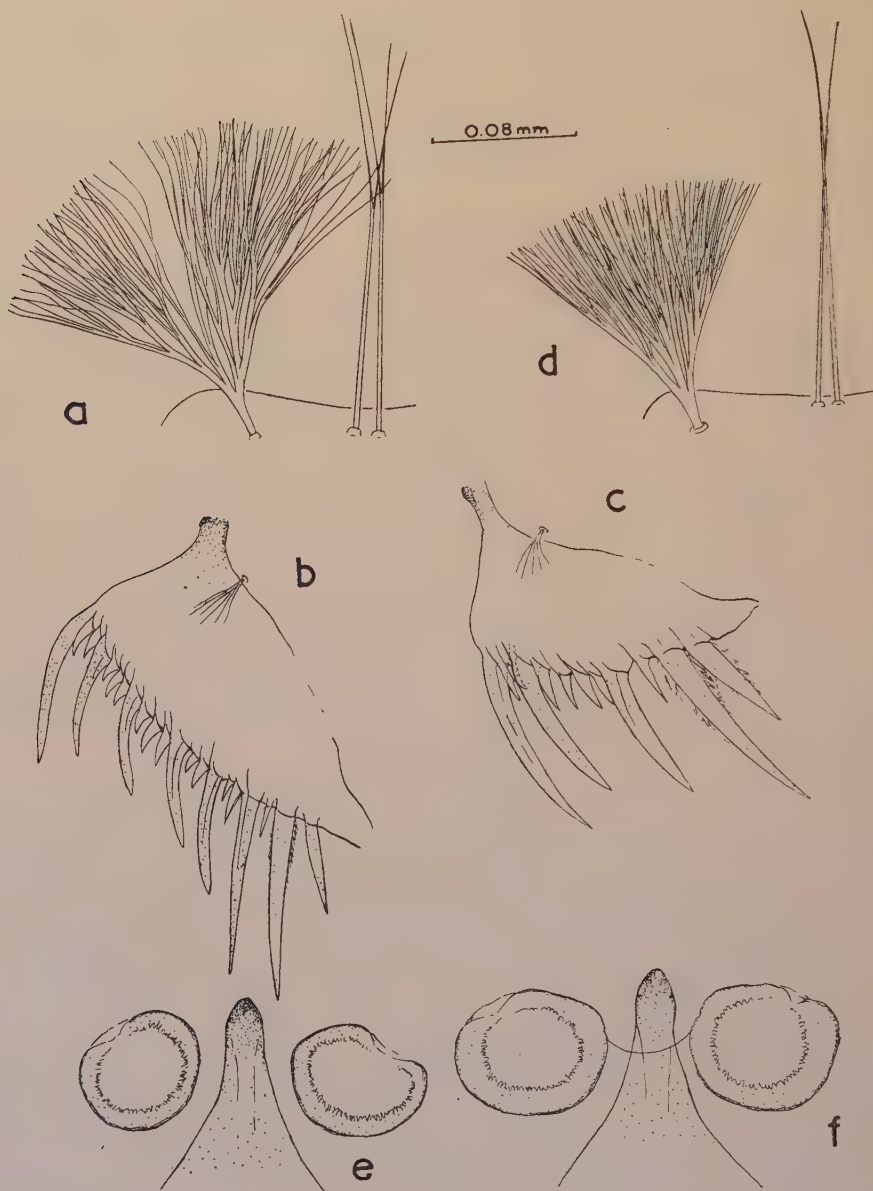


Fig. 8.—Larval structures: *sinensis*; (a) inner and outer clypeal hairs; (f) spiracles: *lesteri*; (b) pecten; (d) inner and outer clypeal hairs: *indiensis*; (c) pecten; (e) spiracles.

Notes.

Comparison of Malayan specimens with those of *sinensis* from Hong Kong which is not far from Canton, the type locality, seems to show that they are conspecific. The adult females agree in all important characters except that the Chinese specimens commonly show some pale scales on the basal third of the costa. This character, however, seems to be connected with latitude and is probably a cline. Only a very few Malayan specimens show small traces of pale scales here usually around or proximal to the humeral cross vein. About one-third of specimens from Assam

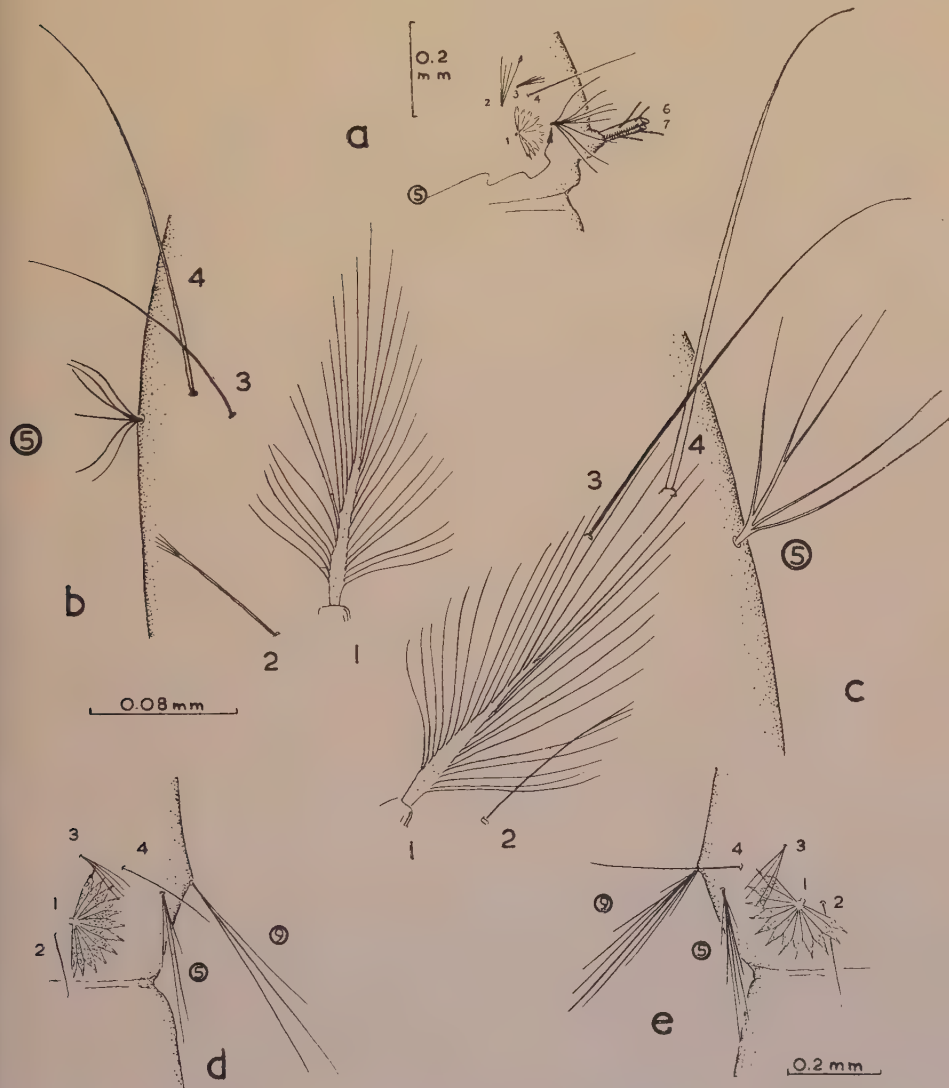


Fig. 9.—Larval structures; the hairs are shown in the positions in which they are usually seen in living larvae: *lesteri*; (a) 2nd abd. segment, right side, to show hair 5: *peditaeniatus*; (b) mesothorax, left side, to show hair 5: *sinensis*; (c) mesothorax, right side, to show hair 5; (e) 6th abd. segment, left side, to show hairs 5 and 9: *nigerrimus*; (d) ditto, right side.

show some pale scales and three-quarters or more of those from Hong Kong. In the latter the scales may be numerous and spread along the costa towards the Sc pale spot. Specimens from further north than Hong Kong all had these scales. Other minor geographical differences are a somewhat darker wing in Malayan specimens, rather less pale scales on the coxae and elsewhere than in those from China, and some differences in larval chaetotaxy (Table IV). An important similarity is in the eggs. Baisas and Hu (1936, Pl. 2, figs. 5, 8), examined eggs of *sinensis* from Hong Kong and found them all of one distinctive type which they called "wide-decked" having a deck about one-third to one-half the width of the whole egg. No other *hyrcanus* eggs examined by them had decks more than one-fifth as wide as the egg. Malayan *sinensis* have closely similar eggs, with decks one-third the width of the egg (fig. 13a), and unlike those of any other Malayan *hyrcanus*. The pupae of Malayan and Chinese *sinensis* have a very similar chaetotaxy, a point that was emphasised by Crawford. Hair V.5 has 9-24 branches in Malayan specimens, and 5-24 in the Chinese specimens described by Baisas and Hu. A minor point of similarity is in the colour of the larvae. Lamborn (1922a) remarks that all *hyrcanus* larvae he saw in China and Japan were green, unlike those of Malaya which showed a wide variety of colours. The larvae of Malayan *sinensis*, however, are usually green (p. 57); the dark colours and more varied patterns belong to the other forms.

If it is accepted that Malayan *sinensis*, though showing some geographical differences, are conspecific with those from Hong Kong, then both may be written *sinensis* Wiedemann, for there seems no reason to doubt that those from Hong Kong represent the type form. Admittedly there is evidence that two forms are included under the name *sinensis* in China (see p. 52), but it appears that one of these is a northerly palaearctic form absent or uncommon as far south as Hong Kong.

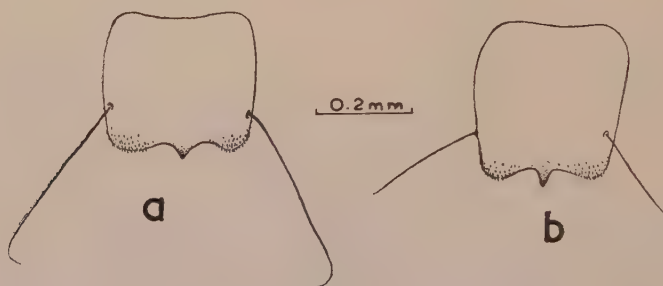


Fig. 10.—Larval structures, saddle and saddle hairs: (a) *argyropus*, (b) *indiensis*.

Distribution.

From the specimens seen the distribution appears to be Assam, Burma, Siam, Indo-China, and China at least as far north as Nanking, with a southwards extension into the Malay Peninsula and Sumatra. It is not known from India proper, Assam being the westerly limit, and possibly it does not extend farther eastwards into the archipelago than Sumatra and the Malay Peninsula. Colless says "There is no definite evidence yet of the occurrence in Borneo of the true *sinensis*" (McArthur, 1950, appendix IV, footnote No. 1).

Type locality: Canton, China.

Type: ♂ and ♀ described; type in Vienna Museum (Yamada, 1924, p. 229). Plesiotypes from Malaya with larval and pupal skins placed in the British Museum (Nat. Hist.), London.

Specimens seen.—ASSAM : (B.M.), Ukhrul, Manipur, 6,400 ft., 1908, 1 ♀ (Pettigrew). (L.S.H.T.M.), Shillong, ix.1921, 6 ♀, 3 ♂ (McCombie Young).

BURMA : (L.S.H.T.M.) Shwenyaung, 1928, 2 ♂ (Feegrade).

SIAM : (B.M.), Patani Cape, 1916, 1 ♀ (H.C. Robinson and N. Annandale). (L.S.H.T.M.), Chiangmai, viii. 1920, 1 ♂ (M.E. Barnes). (I.M.R.), Bangkok, xi.1915, 1 ♀.

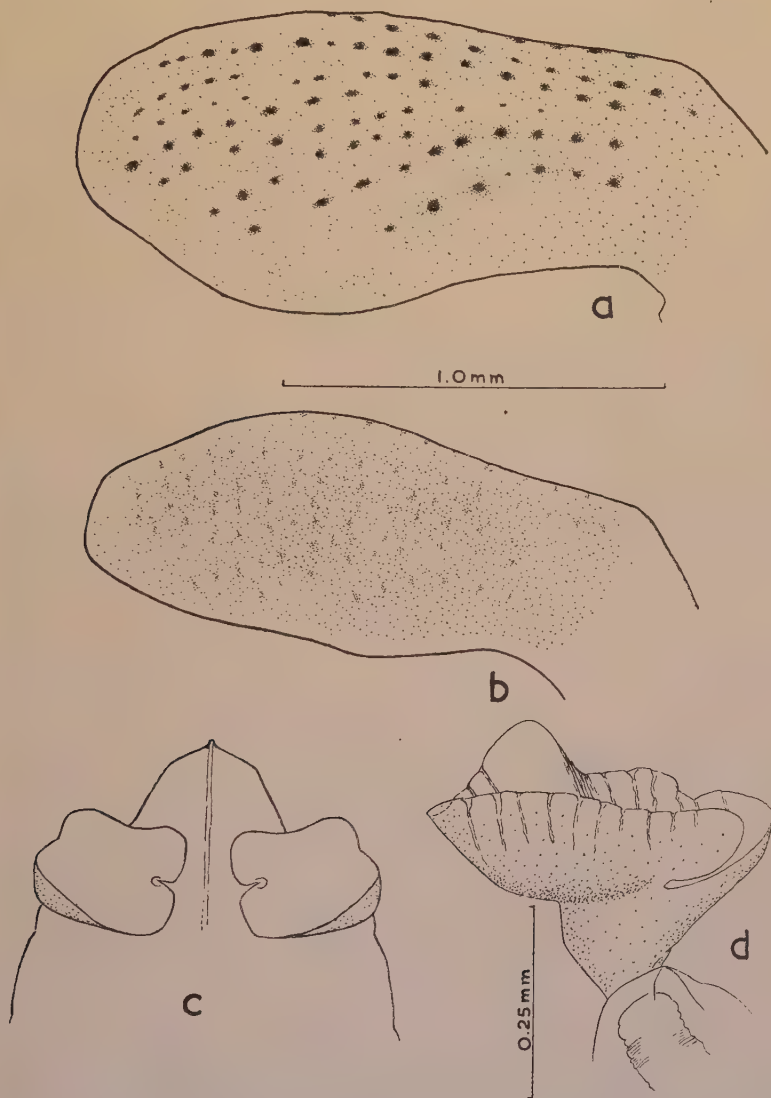


Fig. 11.—Pupae : (a) wing case of *sinensis* to show pattern ; (b) ditto *indiensis* ; (c) living pupa of *nigerrimus* from above to show position and shape of expanded trumpets ; (d) *argyropus* left trumpet as seen in a mounted skin.

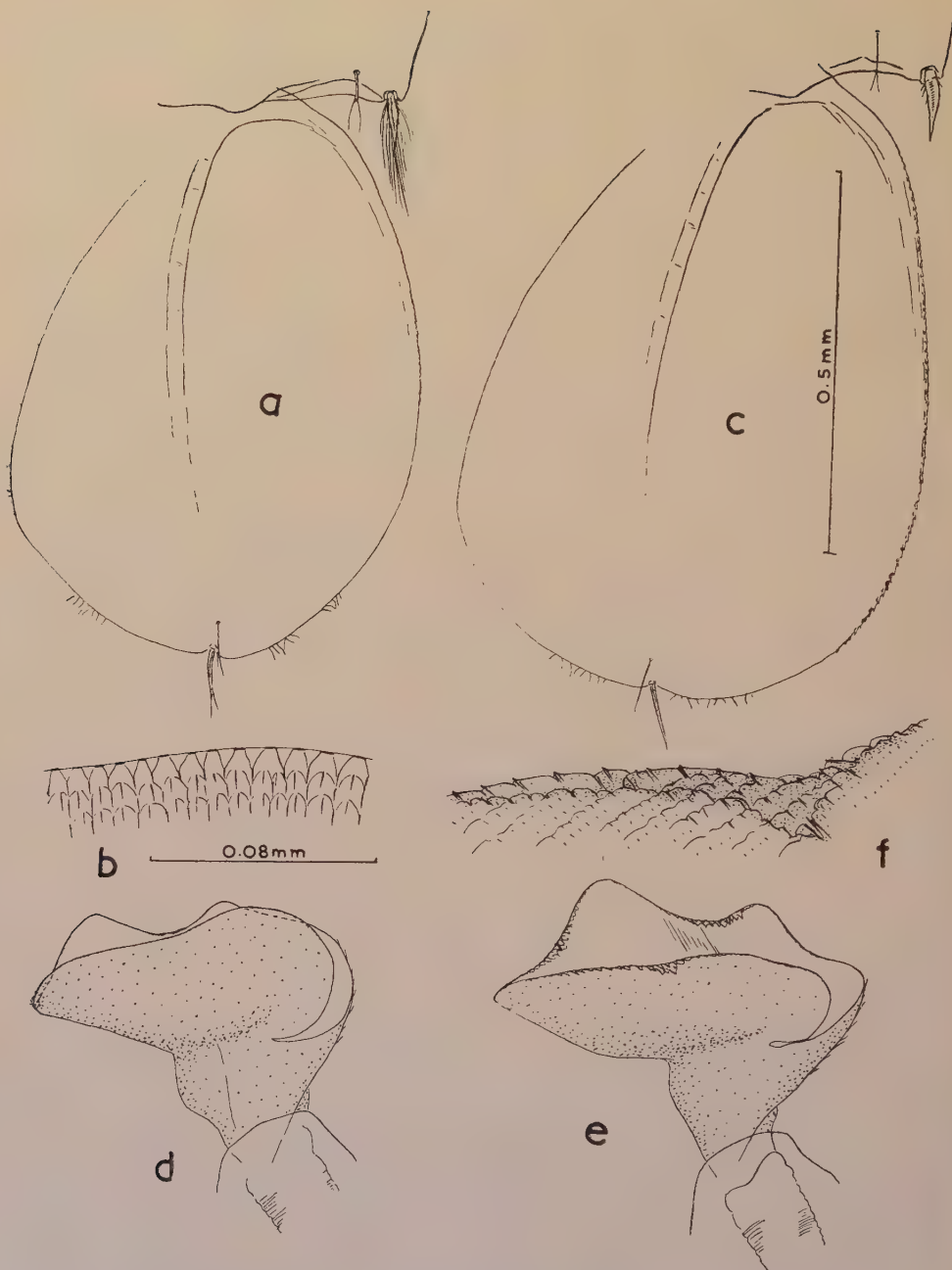


Fig. 12.—Pupae : *sinensis* ; (a) paddle and 8th lateral spine ; (b) portion of rim of trumpet highly magnified ; (d) left trumpet of a mounted skin : *peditaeniatus* ; (c) paddle and 8th lateral spine : *lestevi* ; (e) trumpet ; (f) portion of rim of trumpet.

INDO-CHINA : (B.M.), Saigon, 1914, 1 ♀ (Stanton) ; Tonkin, Cho Ganh, 1922, 1 ♀ (J. A. Lestage) ; Tonkin, 1931–32, 5 ♀, 2 ♂, (Toumanoff). (P.), Saigon, 4 ♀, 3 ♂ (Borel) ; 1932–34, many specimens (Toumanoff).

CHINA : (B.M.), Hong Kong, 1914, 5 ♀ (Dr. H. McFarlane) ; Fukien, Amoy, Yung Chun, 1914, 5 ♀ (Dr. J. P. Maxwell) ; Foochow, 4 ♀ (T. Rennie) ; Chungking, 1 ♀ ; Chekiang, Hang Chow, 1909, 2 ♀ (Dr. C. E. Cornford), Shaohyling, 1902, 6 ♀ (Cornford), Huchow, 1 ♀ (Dr. H. E. Meleney) ; Chusan Is. Tinghae, 1899, 1 ♀ (P. de La Garde) ; Shanghai, 1921, 1 ♀ (W. A. Lamborn) ; Nanking, 1927, 1 ♀, ? northern form (Meleney) ; Kiangsu, Suchowfu, 1 ♀ (Meleney) ; Shantung, Tsinan, 1925, 1 ♀, ? northern form (Maj. W. S. Patton), 1 ♀ (E. Hindle) ; Manchuria, Mukden, 1936, 1 ♀, 2 ♂, ? northern form (Chin Yao-Ting). (L.S.H.T.M.), Hong Kong, 1938, 4 ♀, 56 ♂ (Jackson) ; Yachow (? Yachowfu, 2000 ft., Szechwan), ix. 1937, 1 ♀, ? northern form (R. Crook) ; Nanking, ix. 1933, 3 ♀, ? northern form (D. Yao). (P), Nanking, x. 1933, 1 ♀, 1 ♂ (Central Field Health Station).

MALAY PENINSULA : (B.M.), Taiping, 1899, 1 ♀ (L. Wray). (I.M.R.), many specimens from Kedah, Penang and Province Wellesley in the north to Singapore in the south and Pahang in the east.

SUMATRA (B) : Kotta Radja, 4 ♀, 1 ♂ ; Lokop, 2 ♀.

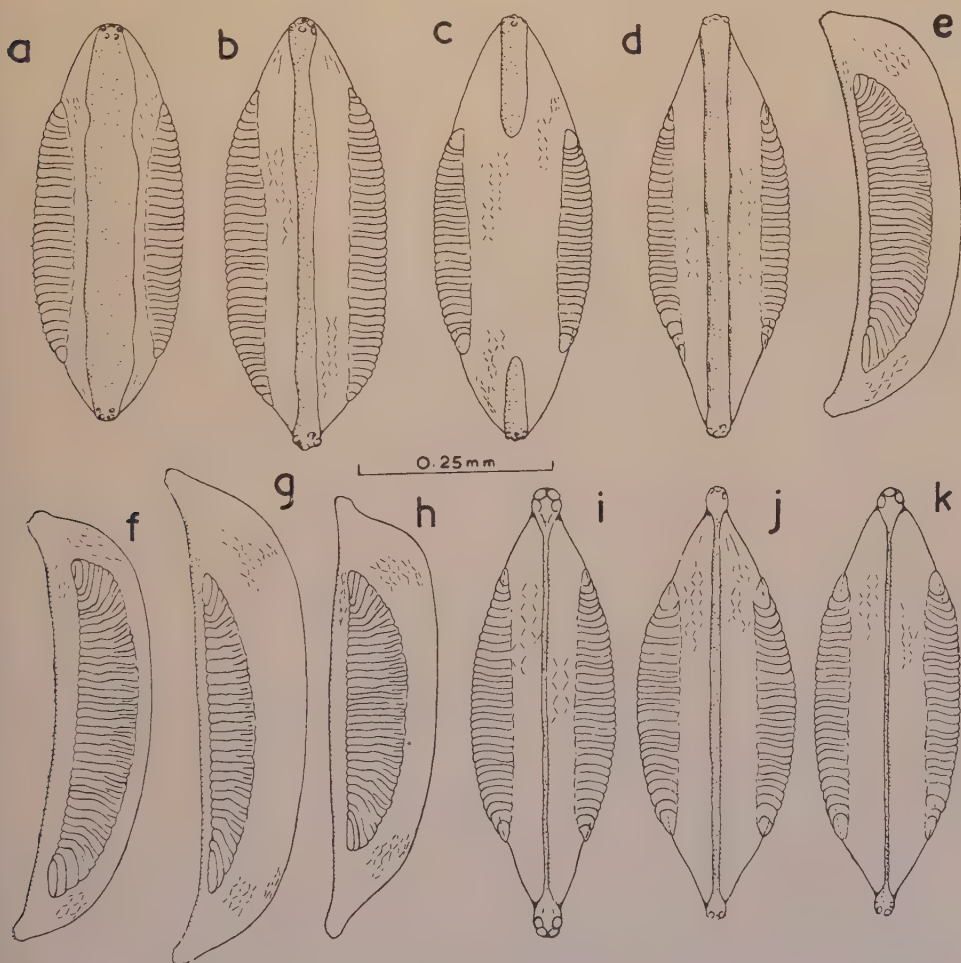


Fig. 13.—Eggs, the head end of each egg is towards the top of the page : *sinensis* (a) from above ; (e) in side view : *nigerrimus* (b, f) : *indiensis* (c) : *peditaeniatus* (d, g) : *argyropus* (h, i) : *lesteri* (j) : *crawfordi* (k).

Anopheles (A.) nigerrimus Giles, 1900.

(nec *nigerrimus* of Baisas & Hu, 1936 and Venhuis, 1939 (= *peditaeniatus*).)

A. ? nero Doleschall, 1851, Java, type, none (*vide* Christophers, 1933, p. 145).

A. bentleyi Bentley, 1902, Tezpur, Assam, type unknown.

A. ? pursati Laveran, 1902, Pursat, Cambodia, type in Institute Pasteur, Paris.

A. ? williamsoni Baisas & Hu, 1936, described from larvae and pupae from Penang, Malaya ; ? material in the Dept. Health, Manila, P.I.

var. X Venhuis, 1939, Java ; type, none.

venhuisi Bonne-Wepster, 1951.

pupal type D of Crawford, 1938.

Adult Female.—In type of wing markings, appearance of the mesonotum, etc., much resembles *sinensis* but in general is rather darker, differs as follows :—

Head.—Proboscis seldom (3/20) with any sign of pale scales before the labella. Palps in general with rather more pale scales, the last two pale bands more often confluent (15/50), first two bands always present (50/50), median basal pale scales about the same, but rather more specimens with scattered pale scales between the pale bands (30/50). Vertex between the eyes dorsally, a little wider.

Thorax.—Setae : propleural, 5–10, av. 7.5.

Legs (fig. 3 d).—Mid and hind femora less often with a pale fleck at the tibial joint. Tarsal pale bands very similar to those of *sinensis*, except that the third and fourth hind tarsal bands are longer and nearly always extend on to the base of the next segment. Fore tarsi ; second band the longest, 2.7–3.1 times as long as wide, average 2.2, rarely extending on to the third segment, seldom any trace of a fourth pale band. Mid tarsi ; second pale band 1.4–2.4 times as long as wide, average 2.2, no fourth band. Hind tarsi ; third and fourth bands longer than the first and second, third usually longest (17/20), first shortest. Third band varies from 3–6.5 times as long as wide, average 3.7, when it is about equal in length to the fifth tarsal segment, nearly always (19/20) it extends on to the base of the fourth segment.

Wings (fig. 1 b).—Length 3.06–4.26, average 3.82 mm. Costa usually (39/50) with a few scattered pale scales dorsally between the level of the humeral cross vein and the sector pale area, numbers vary from 0 to 18, commonly 4–6, never forming a humeral pale interruption. Humeral cross vein with a tuft of dark scales (50/50). Scales of the remigium, especially the anterior border, usually mainly dark (47/50) sub-costal pale area usually well developed, but seldom (1/50) quite complete on vein 1, from which it is occasionally (2/50) absent. Vein 1 ; maximum number of scattered pale scales between remigium and sector pale area rather lower than in *sinensis*, about 20–40 which is about $\frac{1}{4}$ – $\frac{1}{3}$ of the scales pale, preapical dark mark often (30/50) without any scattered pale scales, maximum number about 20. Preapical pale area usually well developed, but occasionally (8/50) small or absent on the costa, or sometimes absent on 2.1 (28/50), always complete on 1. Apical fringe spot wide, when widest it extends from above the termination of vein 1 (21/50) to beyond 3 (47/50), when narrower from 1 to 3. Vein 4 ; stem to r-m mainly dark, sometimes the distal $\frac{1}{3}$ paler, usually a dark mark at r-m, between this and the fork mainly pale ; 4.1 between basal and apical dark mark sometimes (19/50) without dark scales, 4.2 less often so (13/50). Vein 5 ; dark scales at extreme base often form a small dark mark, pale area often well defined, basal dark mark long, 0.27–0.53 mm. usually (48/50) separated by less than its own length from the upper dark mark on 6 ; 5.1 averages rather fewer dark scales between the second and apical dark marks, varying from about 0 to 20. Pale fringe spot present at termination of 5.2 in 68 per cent. (34/50).

It seems that compared with *sinensis*, *nigerrimus* tends to have fewer pale scales on vein 1, and the stems of 2 and 4 may be darker, whilst the dark remigium and longer basal dark mark on 5 add to this tendency for the anterior field of the wing in *nigerrimus* to appear darker. The distal posterior field may appear lighter as scattered dark scales on veins 3-5.1 tend to be fewer.

Abdomen.—Darker. Ventral tuft of dark scales on sternite VII as usual, and occasionally (10/50) a few on sternite VI. Postero-lateral angles of tergite VIII (tip of the abdomen on each side) nearly always (48/50) with a few narrow scales.

Adult Male.—The palps (fig. 4 a) have a well-marked pale band on the base of segment 3 (14/14) absent in *sinensis*, otherwise the markings are similar. Sternite VIII more frequently (7/10) bears a few pale scales, and these are numerous on the apparent dorsal surface of the coxites. *Terminalia* (fig. 6 b).—Only 2-3 pairs of leaflets, usually 2, the pairs somewhat similar in size, fairly broad, with small teeth mostly in the distal half, apparently no basal teeth. The larger leaflet may have teeth on both edges. Ventral lobe of the harpago with 2 setae.

Larva.—Differs from the larva of *sinensis* mainly as follows. Macroscopic appearance, green, brown or black, usually with a pale mark on the prothorax and pale bands on abdominal segments III, V and VIII, that on III the best developed. Black larvae with well-developed white bands appear vividly marked.

Head (fig. 7 a, e).—Inner anterior clypeal hairs nearly always simple, outer ones with about 70-85 branches, usually stiffer, blacker and a little shorter than those of *sinensis*. Sutural with a large number of dark branches, 14-24, average 17. The antenna is stout compared to that of some of the other forms such as *peditaeniatus* and *argyropus* and occasionally very darkly pigmented. Adults resulting from larvae with very dark heads and antennae do not appear in any way unusual. The antennal shaft hair usually reaches beyond the end of the shaft. *Thorax*.—Inner shoulder hair usually with 2-4 small branches towards the tip, less often simple. Mesothoracic hair 5 with 5-10 branches. *Abdomen* (figs. 7 c, 9 d).—Palmate hairs with less slender tips, pigmentation usually darker, average diameter slightly less (see Table III, p. 49). Hairs 5 and 9 with a smaller number of branches on most segments; hairs 5 on V with 2-4; segment VI, hair 5, with 2-5 branches, hair 9, 2-4. Tergal plates on VIII of the same shape as *sinensis*, but average size slightly smaller; ratio length/width 0.56-0.68, average 0.62. Pecten with fewer, 6 or 7, long teeth.

Pupa (fig. 11 c).—Trumpet similar to that of *sinensis*; differs in other respects mainly as follows. Pigmentation moderate to dark, with the base of the paddle, a band on the ♂ genital plate, and often the tips of the antennal cases, dark. Wing cases with a vague mottled pattern of veins and cross bars. Hairs 2 and 5 with more branches; segment V hair 2 with 7-20, commonly about 12 branches, hair 5 a strong dark tuft composed of 40-60 branches. Cuticular denticles often very small so that lateral borders of segments may not show serrations. Paddle border commonly a little less convex, refractile border 0.63-0.71, average 0.67, the length of the paddle.

Egg (fig. 13 b, f).—With a much narrower deck than *sinensis*, about one-seventh the width of the egg, but still concave in side view, though less so. Floats longer with about 35-40 ribs.

For other illustrations of this species see: *Adult* ♀, Christophers, 1933 ? fig. 24/1; Hodgkin and Rajamoney, 1933, Pl. 1, figs. 2 b, 3 b (♂ palp); Venhuis, 1939, fig. 3; *larva*, Hodgkin and Rajamoney, ? Pl. 1, fig. 4, ? Pl. 2, figs. 2, 3; Venhuis, figs. 4, 5; *pupa*, Hodgkin and Rajamoney, ? Pl. 2, figs. 4, 5; Venhuis, fig. 2; Crawford, fig. 13; *egg*, Walch and Walch-Sorgdrager, Pl. III, figs. 31-33; Christophers, 1933, ? Pl. 1, figs. 2, 3.

Identification.—Much resembles *sinensis* in general features such as size, markings of the mesonotum and overall wing pattern. Characterised chiefly by the hind tarsal pale bands, especially the third, spreading slightly across the joints, long basal dark mark on 5, a few scattered pale scales usually present towards the base of the costa, dark scales on the humeral cross vein, and generally a few at the tip of the abdomen on each side (8th tergite). In about five per cent. of Malayan specimens the third hind tarsal band does not extend on to the base of the fourth segment. Additional characters are: subcostal pale spot rarely complete on vein 1, remigium usually mainly dark, chitin of abdomen darker than *sinensis*, vein 1 often without pale scales between Sc and pre-apical pale spots, numerous propleural setae, 5–10, usually 7 or more, and well-developed pale scales on the mid coxa. The palps of the male have a pale band at the base of the third segment. The larvae, like those of *sinensis*, differ in a general way from the other species in the large palmate hairs, large wide 8th tergal plate, and rather bigger spiracles. Points not mentioned in the key are the transverse white bands commonly present on the abdomen which is often green or black. The antennae are rather stout with a large shaft hair, and the palmate hairs are usually a little smaller and more darkly pigmented than those of *sinensis*, and the outer clypeals have somewhat stiffer branches. The combination of a large number of branches on the sutural hair, and the low number of hairs 5 and 9 on the abdominal segments, is distinctive of this species (but see p. 44, species D2).

Notes.

Identity of the type.

Although the type specimen of *nigerrimus* Giles from Calcutta is badly damaged there is little doubt that it is the species described here as *nigerrimus*. The hind legs, abdomen, palps and one wing are missing, but the following points can be seen. The wing has the dark blurry type of markings, the subcostal pale spot is well developed, there are few pale scales on the base of vein 1, the remigium is mainly dark, the humeral cross vein bears a tuft of dark scales, the basal dark mark on 5 is fairly long, ending a short way above the upper dark mark on 6 which is fused with the lower one, and there are 8 propleural setae on one side. The presence of the clypeal scale tufts confirms that the specimen is a form of *hyrcanus*. The blurry type of wing pattern and the large number of propleural setae rules out *indiensis* or *crawfordi*. *A. sinensis* is not found nearer Calcutta than Assam and Burma, and the long basal dark mark on vein 5 of the type excludes it. Although the fusion of the dark marks on 6 is commoner in *peditaeniatus* than in *nigerrimus*, and on grounds of probability the former is the most likely alternative, the characters of the type, in particular the dark remigium, scale tuft on the humeral cross vein, scarcity of pale scales on the base of 1 and the numerous propleural setae, seem clearly to exclude *peditaeniatus*. *A. argyropus* can be ruled out on grounds of improbability, and because the type has too many propleural setae and a well-developed Sc pale spot. *A. lesteri* can be excluded because of the numerous propleural setae and the scales on the humeral cross vein of the type, and because there is no evidence at present that the range of *lesteri* extends to India. Provided, therefore, that there is no undescribed form common round Calcutta which I have not seen, a process of elimination identifies the type, the characters of which so far as they can still be made out, agree well with those of the form described here as *nigerrimus*. Furthermore I have seen two typical specimens of this form from Calcutta (a male and female in good condition in the Amsterdam Museum), and others from elsewhere in the Indian area. There are certain points of discordance in the descriptions of Giles (1900) and Theobald (1901), but these can readily be explained by the bad condition of the specimens noted by Theobald. Both authors describe the tips of the palps as black, but I agree with James and Liston (1911) that they must

have been misled by an appearance due to damage ; the tips of the palps are always pale in Oriental *hyrcanus*. Theobald states that the apical fringe of the wing is black, but in fact it is largely missing.

Anopheles nero (Dol.) (*Culex nero*).—Edwards regarded this as probably *hyrcanus*, and Christophers followed him placing it under var. *nigerrimus*. The brief description and vague figure given by Doleschall are quite inadequate to allow a positive identification, but since *nigerrimus* Giles seems to be the commonest member of the *hyrcanus* group in Java, it is reasonable to leave the name *nero* as a query synonym.

Anopheles bentleyi Bentley.—The description and figure seem to point to *nigerrimus* or *peditaeniatus*. The wing pattern is stated to be blurry which excludes *indiensis* or *crawfordi*, and the upper dark mark on 6 is too long for *sinensis*. The first longitudinal vein is described as chiefly black scaled with here and there white scales, the hind tarsal bands appear to be narrow ; this seems to indicate *nigerrimus*, rather than *peditaeniatus* in which vein 1 usually bears numerous pale scales, especially in specimens from the Indian area, and in which the hind tarsal pale bands are often broad.

Anopheles pursati Lav.—The identity of the types of this species has already been discussed (Reid, 1947). Although *pursati* may eventually prove not to be *nigerrimus*, it is convenient for the present to treat it as a synonym. To make a positive identification, the two type specimens will have to be removed from the balsam in which they are mounted. This can be done, but is best postponed until the *hyrcanus* group in Indo-China has been more carefully studied ; *sinensis* from that country seem very variable.

Variety *williamsoni* Baisas & Hu.—Baisas and Hu (1936) described variety *williamsoni* from larvae and pupae from Penang, Malaya. The large number of branches (19–20) on the sutural hair of the larva shows that *williamsoni* can only be *nigerrimus*, *indiensis*, *argyropus* or a mixture of these. It cannot be *argyropus* because the pupal paddle is described as like that of *sinensis* from China and no comment is made on spine VIII, whilst *argyropus* has a long refractile paddle border and spine VIII without or with much reduced branches as in *peditaeniatus*, in which both these characters were noted by Baisas and Hu. It is less easy to decide between *nigerrimus* and *indiensis*, but if the larval hair counts for these species and *williamsoni* are tabulated and compared it is found that for nine hairs distributed on the head, thorax and abdomen, *williamsoni* agrees more closely with *nigerrimus* than with *indiensis* for seven of the nine.

Variety X.—Venhuis (1939) accurately described *nigerrimus* from Java and elsewhere in Indonesia under the provisional name of *A. hyrcanus* variety X. The name *nigerrimus* was employed by him in the same sense as Baisas and Hu for what is here called *peditaeniatus*. Venhuis' description included a photograph of a wing which can only be that of *nigerrimus*—it clearly shows the long basal dark mark on 5 and a fringe spot at 5.2. His description of the larva very closely fits that given here and he illustrates the stout antenna, sometimes very darkly pigmented. The agreement in the hair counts for the three hairs of most value in distinguishing *nigerrimus* larvae, is remarkably close.

	Head sutural	Abdomen 5	Seg. VI 9
Venhuis, Indonesia	13–23	3–5	3–4
Present account, Malaya ...	12–24	2–5	2–4

Bonne-Wepster (1951) has proposed the name *venhuisi* in memory of Dr. W. G. Venhuis, to replace var. X. I very much regret having to treat this name as a

synonym, for I knew Dr. Venhuis personally and admired his work. If further study shows that the variation I noticed in *nigerrimus* from Java (see below) is of sub-specific rank, the name *venhuisi* is available.

Relation to A. pseudosinensis Baisas & Hu.

Through the kindness and courtesy of Dr. de Leon and Mr. Baisas I have been able to examine a series of specimens of *pseudosinensis* including paratypes, complete with larval and pupal skins. Whilst this form is intermediate in many respects between *nigerrimus* and *sinensis*, it is closer to *nigerrimus*, and the two were probably derived from a single ancestral form (p. 53). The adults of *pseudosinensis* resemble *nigerrimus* more than *sinensis* in wing pattern, the basal dark mark on 5 is usually rather long, though not as long as in *nigerrimus*. The basal third of the costa sometimes has a few scattered pale scales like those of *nigerrimus*. Only about 10 per cent. have a pale fringe spot at the termination of 5.2 thus differing somewhat from *nigerrimus* and *sinensis*. Occasionally there are a few scales at the tip of the abdomen (tergite VIII). The males show two important affinities with *nigerrimus*; there is a short pale band on the base of the third palp segment, and the phallosome has only two pairs of leaflets very similar in shape to those of *nigerrimus* and with teeth on both edges. The club on the dorsal lobe of the harpago, however, seems to be more like that of *sinensis* without a markedly projecting spine. The hind tarsi of the male sometimes show a narrow basal band on segment 5. Larval and pupal chaetotaxy show an affinity with *sinensis* in many, but not all points (see Table IV). The general features of these stages, large palmate hairs, large wide tergal plate on VIII, and pupal trumpet with a simple rim, clearly place *pseudosinensis* with *sinensis* and *nigerrimus*. Table IV shows that in the larval chaetotaxy of the head and thorax, *pseudosinensis* resembles *sinensis* for all the hairs that have been enumerated, especially the sutural and transsutural. On the abdomen, however, the resemblance is to *nigerrimus*, especially in the low number of branches on 5 and 9 of VI. The large abdominal hairs, 2 and 5, on the pupa have a small number of branches as in *sinensis*, but the terminal paddle hair, like that of *nigerrimus* is usually simple. The width of the deck in the egg is intermediate between that of *sinensis* and *nigerrimus* and does not overlap with them. In view of the fact that other forms, such as *peditaeniatus* and *lesteri*, which vary geographically nevertheless seem to remain fairly constant in most of their important characters, it appears that *pseudosinensis* should be regarded as a distinct species.

Insufficient material of *nigerrimus* from outside Malaya has been examined to study variation, but many specimens in a series from Java were very dark in wing, thorax and abdomen, much resembling *argyropus* in this respect. It seems to be a common species in Java as it formed a large proportion of the specimens seen from there, and was the only species encountered by Venhuis among hundreds of specimens examined from east Java; also out of 15 batches of eggs examined by Walch and Walch-Sorgdrager (1935), all but a very few seem to have been those of *nigerrimus*. Their excellent drawings leave no doubt of this, for their figs. 31-33 accurately show the important features; long floats with many ribs, rounded ends, deck about one-seventh as wide as the egg and concave in side view, contrasting with their fig. 34 which is clearly that of the egg of *peditaeniatus*, tapering to more pointed ends and with short floats with much fewer ribs. The Indian egg illustrated by Christophers and Barraud (1931) also seems to be that of true *nigerrimus*. Walch and Walch-Sorgdrager found 22-41 float ribs with an average of 32.6; Christophers and Barraud give 30-35. My figures are somewhat higher, 35-40, but are based on counts of only five specimens.

Distribution.

Ceylon, India (Central Provinces and Bengal), Assam, Burma, Siam, Malay Peninsula, Sumatra, Java, Borneo, Celebes, Moluccas (Boeroe). I have

seen only one male specimen from Borneo, but Kariadi (1941) found it (var. X Venhuis) common at Martapoera in south-east Borneo.

Type locality : Calcutta, India.

Type : ♀ in Brit. Mus. Plesiotypes from Malaya with larval and pupal skins placed in Brit. Mus.

Specimens seen.—INDIA : (B.M.) Calcutta, iv.1899, 1 ♀ (the type) (Dr. C. W. Daniels), Central Provinces, Bihar, Kiepur, ix.1915, 1 ♀ (India Museum). (A), Calcutta, xii.1907, 1 ♀, 1 ♂ (India Museum). (I.M.R.), 1 ♀. (S), 2 ♀, 1 ♂.

CEYLON : (B.M.), 1907, 1 ♀ (Galle). (L.S.H.T.M.), Anuradhapura, ii. 1938, 1 ♀ (Buxton). (S), 2 ♀, 1 ♂.

ASSAM : (L.S.H.T.M.), Jorhat Province, 14 ♀ (Dr. Hermette) ; Mangaldai, 1934, 1 ♀ (Assam med. Res. Soc.) ; Dhubri, 1934, 2 ♀ (Assam med. Res. Soc.). (A), Sylhet, 1905, 1 ♀ (Maj. Hall).

BURMA : (I.S.H.T.M.), Shwenyaung, 1928, 1 ♀ (Feegrade).

SIAM : (L.S.H.T.M.), viii.1920, 1 ♀ (M. E. Barnes). (S) 1 ♀.

MALAY PENINSULA : (I.M.R.), Many specimens, from North Kedah, Penang and Province Wellesley in the north to Johore in the south.

SUMATRA : (B.M.), Fort de Kock, 1920, 1 ♀ (E. Jacobson). (B), Mandailing, 5 ♀, 1 ♂ ; Rao, 2 ♀, Banka, 2 ♀, 1 ♂.

JAVA : (B), Tjilatjap, 14 ♀ ; Tasik Malaya, 10 ♀ ; Tanggarong, 14 ♀ ; Soerabaia, 1 ♂ ; Kapetakan, 13 ♀. (S), Batavia, xi.1933, 2 ♀ (R. Soesilo).

BORNEO : (B. M.), Sarawak, ? Kuching, 1914, 1 ♂ (J. C. Moulton).

CELEBES : (B), Makasser, 1 ♂.

MOLUCCAS : (B), Boeroe, 2 ♀.

Anopheles (A.) indiensis Theobald, 1901.

Pupal type C of Crawford, 1938.

Adult female.—A distinctive form, with a bright sharp wing pattern, pale scales on the costa and well-marked eye spots on the thorax. Differs from *sinensis* mainly as follows.

Head.—Proboscis, mean length in five specimens 1.91 mm., ratio fore femur/proboscis 0.86. Palps with the first and second pale bands usually well marked, always present, as are the basal median pale scales ; scattered pale scales between the bands usually present (41/50) and often numerous.

Thorax.—Eye spots of the mesonotum more conspicuous mainly because of lesser development of other dark areas and contrast with more marked grey pruinosity. Propleural setae 2-5, average 3.7.

Legs (fig. 3 e).—Coxal pale scales well developed, usually only one upper mid coxal seta (19/20). Tarsal pale bands longer. Fore tarsi, second band the longest, 3.2-8.3 times as long as wide, average 3.4, usually between $\frac{1}{3}$ and $\frac{1}{2}$ the length of segment two, occasionally (8/50) spreading slightly on to segment three ; sometimes there is a minute fourth pale band (19/50). Mid tarsi, second band 1.4-3.8 times as long as wide, average 2.2. Hind tarsi always with four pale bands of which the 3rd is the longest and always (20/20) extends on to the base of the fourth segment, the other bands less often extend across the joints, 1st (0/20), 2nd (9/20), 4th (13/20) ; third band 4.8-7.6 times as long as wide, average 5.8 ; 1.1-1.6 times as long as the fifth segment ; in specimens with the broadest hind tarsal bands the fourth segment may be largely white.

Wings (fig. 2 a).—Brightly marked, with light and dark scales well contrasted and short discrete dark marks on the posterior veins. Length 2.86-4.00, average 3.46 mm. Basal half of costa always with pale scales, some of which usually (48/50)

form a small humeral pale interruption often slightly distal to the humeral cross vein, the remainder are scattered between this and the level of the middle dark mark on vein 1 varying in number from about 6-40 and averaging about 15-20, exceptionally this part of the costa may be almost completely pale and scattered pale scales may extend as far as the subcostal pale area. Occasionally (8/50) there may be a few pale scales proximal to the humeral cross vein which bears a small tuft of dark scales (50/50). Remigium with the scales of the anterior border mainly pale. Subcostal pale area well developed but usually (39/50) incomplete on vein 1. Markings on vein 1 very similar to those of *sinensis*, though pale scales on preapical dark mark average slightly less varying from 0 (4/50) to about 20-40 which is about $\frac{1}{4}$ of the scales pale, tip of the vein usually dark (45/50). Preapical pale area present on the costa (50/50), occasionally (4/50) incomplete or absent on 2.1, slightly more distal in position than in *sinensis*. Apical fringe spot tends to be shorter, commencing lower down at (17/50), or below vein 1 (33/50), ending as usual beyond 3. Veins 2, 3 and 4 with pale areas and dark marks well contrasted, fewer scattered dark or slightly dark scales in the pale areas. Vein 5 pale at the base though sometimes with one or two dark scales at extreme base, this pale basal area followed by the usual dark mark which is short (0.1-0.3 mm.), usually (39/50) shorter than the pale area and separated by at least its own length from the upper dark mark on 6 (49/50). Two upper dark marks on 5.1 always separate, fewer scattered dark scales between second and apical dark marks on this vein, range 1-20, usually about 10. Vein 6 entirely pale except for the two dark marks, the upper of which is often (35/50) the shorter, but less often than in *sinensis*, the apical one is frequently (27/50) equal to the apical dark mark on 5.2 and rather less often (21/50) longer. Pale fringe spot at termination of 5.2 in 82 per cent. (41/50).

Abdomen.—Postero-lateral angles of tergite VIII with a few narrow scales as in *nigerrimus* (24/25), occasionally (2/25) one or two scales on sternite VI in addition to the usual tuft on VII.

Adult Male.—The palps have a pale band at the base of segment 3 (10/10) as in *nigerrimus* though usually somewhat narrower, median dorsal pale scales on segments 2 and 3 often scarce. Usually with a few pale scales on sternite VIII (dorsal) (5/5), and always many on the coxites. *Terminalia* (figs. 5e, 6c).—Only 2-3 pairs of leaflets of which only the first pair bears teeth, no basal teeth. Ventral lobe of the harpago with the usual 2 setae, occasionally only the larger one is distinguishable.

Larva (figs. 8c, e, 10b).—Commonly green, but may be pale brown to blackish, without transverse pale bands on the abdomen. Differs from *sinensis* mainly as follows. *Head*.—Inner anterior clypeal hairs simple, sutural hairs with 11-17, average 13, branches. *Abdomen*.—Palmate hairs smaller, tips less slender, pigmentation generally denser and more uniform. Segment V, hair 5, 5-9 branches, segment VI, hairs 5 and 9, 5-9 branches. Tergal plate on VIII less transverse, usually more than two-thirds as long as wide, and on the average smaller, ratio length/width 0.64-0.77, average 0.69. Pecten with only 5-7 long teeth, usually 6. Spiracles smaller. Saddle hair weak, shorter than the saddle or the width of segment VIII.

Pupa (fig. 11b).—Usually as lightly pigmented as *sinensis*, but with sharply marked dark tips to the antennal cases; occasional dark specimens are encountered with well-pigmented wing cases and paddle bases. Trumpets rather smaller than those of *sinensis* and *nigerrimus*, sometimes with some slight thickening or irregularity of the rim in places. Branches on hairs 2 and 5 about as numerous as in *nigerrimus*: segment V, hair 2 with 9-28, commonly about 12 branches, hair 5 a tuft of 30-50 branches. Paddle, refractile border 0.67-0.80, average 0.75 the length of the paddle.

Egg (fig. 13f).—At once distinguished by the deck which is divided into two areas, one at each end of the egg, each roughly one-sixth its width. In side view the

upper surface is slightly raised or convex, sloping down towards the points, in contrast to *sinensis* and *nigerrimus* in which the upper surface is concave rising upwards to the point, but like these species the points do not project much when viewed from above so that the egg appears rounded at the ends. Floats rather small with about 25–30 ribs.

For other illustrations of this species see Crawford, fig. 12 (pupa).

Identification.—This is one of the easiest species to identify in the adult stage. Distinguished by the sharp wing pattern with short dark marks and scattered pale scales on the basal half of the costa, fairly well marked eye spots on the grey mesonotum, and fairly broad hind tarsal pale bands. Other characters are : usually only one upper mid coxal seta, pale coxal scales well developed, usually a fringe spot at 5.2, and a few scales at the tip of the abdomen as in *nigerrimus*. The larva is not easy to identify, in addition to the characters in the key, hair 13 on abdominal segment IV (on the ventral surface of the abdomen) is not very much longer than the same hair on segment III, nor has it much fewer branches, 6–12, commonly 9. In most of the other species this hair (IV.13) has considerably fewer and much longer branches thus resembling the same hair on segment V, rather than on segment III. In *lesteri* the branches on this hair (IV.13) are about three times as long as those of III.13 and the common number is 5 (range 4–9) ; in *crawfordi* the branches are about twice as long and the common number is 6 (range 4–7). The antennal shaft hair of *indiensis* is variable, but often rather small, not reaching to the end of the shaft.

Notes.

There is not the least difficulty in identifying the specimen which is labelled as the type, with the species described here as *indiensis*. The pale scales on the costa, the sharp wing pattern, the eye spots on the mesonotum, and the broad hind tarsal bands put the identification beyond question. The specimen, however, is labelled in Theobald's writing "*Anopheles annularis* var *alboannulus* (type) Theobald". At that time Theobald thought *annularis* V. der Wulp was close to *sinensis* Wied., and treated it as a subspecies of the latter in the first volume of his monograph (1901). This mistake he corrected in Vol. III (1903, p. 90). This leaves the name *alboannulus* to account for, and Giles (1904, p. 38), who saw the specimens bearing the name, explains that it was a manuscript name, and he sinks it along with *vanus* Walk., *nigerrimus* Giles, *indiensis* Theo. and *minutus* Theo. as synonyms of *sinensis* Wied. (= *hyrcanus*). Theobald described only two subspecies of *sinensis* ; *indiensis* in 1901 (p. 145) and *minutus* in 1903 (p. 91). The type of *minutus*, with locality data that agree with those published by Theobald, is labelled in Theobald's writing "*Anopheles sinensis* sb. sp. *minima* F.V.T.". It seems therefore that both *minima* and *alboannulus*, or *alboannulatus* as Giles, and later Christophers wrote it, are manuscript names which had to be changed because they were preoccupied, and that Theobald forgot to alter the labels under the specimens. The only argument against accepting this explanation and treating the specimen as the type of *indiensis*, is a statement by Giles (1902, p. 306) that "I have not seen the *indiensis* form, as the single specimen from which it was described by Mr. Theobald was a loan from a private collection". But this seems to be incorrect for Theobald states that *indiensis* was described from several specimens, and it seems significant that Giles (1904) saw several specimens of *alboannulatus*. Later there is further confusion when Theobald (1907, p. 86), treats the reference to *alboannulatus* by Giles (1904) and by James and Liston (1904, p. 81, quoting Giles), as a mistake for *albotaeniatus* Theo. 1903. Christophers (1924) follows Theobald. But this may be dismissed as an error of Theobald's and irrelevant to the present aim of identifying the type of *indiensis*. Giles could hardly have confused specimens of *albotaeniatus* with *sinensis*. There seems no reasonable doubt, therefore, that the specimen labelled *alboannulus* is the type

of *indiensis* Theo., and I shall follow Christophers (1924) and Yamada (1924) in treating it as such.

Distribution.

From the specimens seen the distribution appears to be India (Madras), Assam, Burma, Indo-China, Malay Peninsula, Sumatra and Borneo. Presumably it occurs also in Siam, although no specimens were seen.

Type locality: Madras, India.

Type: ♀ in Brit. Mus. Plesiotypes from Malaya with larval and pupal skins placed in Brit. Mus.

Specimens seen. INDIA: (B.M.), Madras, 1 ♀ (the type) (Capt. Cornwall). (S), 1 ♂.

ASSAM: (L.S.H.T.M.), Jorhat Province, 7 (Dr. Hermette); Juri, Sylhet, 1938, 1 ♂ (C. Hamilton). (A), Sylhet, 1905, 1 ♀ (Maj. Hall).

BURMA: (L.S.H.T.M.), Kale valley, 1944, 2 ♀ (T.T. Macan). (I.M.R.), Kale valley, 1944, 1 ♀ (T. T. Macan).

INDO-CHINA: (B.M.), Tonkin, 1931, 1 ♀ (Toumanoff). (P), 1 ♀ 3 ♂ (Toumanoff).

MALAY PENINSULA: (B.M.), Taiping, 1899, 7 ♀ (L. Wray); Kuala Lumpur, 1902, 1 ♀ (Dr. Durham), 1904, 2 ♂ (G. F. Leicester). (I.M.R.), many specimens seen, from Penang, Kedah and Province Wellesley in the north, to Negri Sembilan in the south, and Pahang in the east.

SUMATRA: (B), Sibolga, 8 ♀; Rao, 1 ♀; Banka, 5 ♀, 4 ♂.

BORNEO: (B.M.), Sarawak, ? Kuching, 1914, 1 ♀, 1 ♂ (J. C. Moulton). (I.M.R.), Labuan, vii-ix.45, 4 ♀, xi.48, 1 ♀ (D. H. Colless).

Anopheles (A.) peditaeniatus Leicester, 1908.

A. nigerrimus of Baisas 1931, Baisas & Hu 1936 and Venhuis, 1939.

Pupal type E of Crawford, 1938.

Adult Female.—Not unlike *nigerrimus* in general appearance but distinguishable on a number of points; differs from *sinensis* chiefly as follows:—

Head.—Ornamentation of the palps similar to *sinensis* except that the third and fourth (apical) bands are usually confluent or partly so (47/50), and the scales between the bands often have a lead grey or slightly purple tinge (*cf.* Baisas, 1931 p. 442).

Thorax.—The longitudinal dark marks on the mesonotum well developed and the eye spots subdued. Propleural setae, 2–6, average 4.1.

Legs (fig. 3 f, i).—Pale scales on the coxae usually fewer and less noticeable, tarsal pale bands much longer. Fore tarsi, second band usually (48/50) the longest, varying from 3.1 to 6.0 times as long as wide, average 3.5, equivalent to more than 1/3 to just under 2/3 as long as the second segment: occasionally (8/50) with a minute fourth band, and occasionally (11/50) with minute basal bands. Mid tarsi, third band usually (18/20) the longest, from more than 1/3 to more than 1/2 the length of the segment. Hind tarsi with four pale bands of which the third is the longest and always extends on to the base of the fourth segment, this band varying from 5.1 to 9.4 times as long as wide, average 5.9, equivalent to 1.1 to 1.8 times the length of the fifth segment, and 0.4–0.8 times the 4th segment; the other bands extending across the joints as follows; 1st (0/20), 2nd (3/20), 4th (20/20); compared with *indiensis* it appears as if the bands are better developed distally.

Wings (fig. 2 b).—General appearance not unlike the wing of *sinensis* or *nigerrimus*, but the pale scales on costa and vein 1 tend to be more creamy and less white.

Length, 2.94–3.86, average 3.50 mm. No pale scales on the costa between base and subcostal pale area. Humeral cross vein usually bare (41/50). Remigium with the anterior border always pale, the scales generally (43/50) more or less silvery white and contrasting with the cream scales on vein 1. Subcostal pale area normally developed, seldom (2/50) complete on vein 1 but always present. Pale scales on vein 1 between remigium and sector pale area varying from 2–3 up to $1\frac{1}{2}$ to $2\frac{2}{3}$ of the scales pale, sometimes (14–50) they are mostly in the basal $\frac{2}{3}$ of this section, and this tendency may be much developed in specimens from outside Malaya (see p. 35). Pale scales on the middle dark mark and those of the subcostal pale area and the scattered pale scales distal to this, usually more or less continuous and mixed with a variable number of scattered dark scales; the pale scales distal to the subcostal pale area are usually numerous, up to about $1\frac{1}{3}$ – $1\frac{1}{2}$ of the scales pale. Preapical pale area on the costa usually well developed, occasionally (4/50) small, sometimes (6/50) incomplete or absent on vein 2.1. Apical fringe spot commonly commencing above vein 1 (29/50), otherwise at vein 1, and ending beyond 3, very occasionally (1/50) fusing dorsally with the preapical pale area. Stem of vein 2 dark or mainly dark. Stem of vein 4 to cross vein r–m mainly dark and from here to fork usually (39/50) mainly dark. Vein 5, basal pale area may be well developed and equal to or occasionally longer than the succeeding basal dark mark, or it may consist of only a few scattered pale scales. Basal dark mark varies from 0.3 to 0.5 mm. and is usually (47/50) separated by less than its own length from the upper dark mark on 6, and sometimes (9/50) ends level with this mark. Vein 5.1 between middle and apical dark marks with rather fewer scattered dark scales, 1–25. Vein 6, the two dark marks occasionally (6/50) confluent, the upper sometimes (16/50) equal to or longer than the apical. No pale fringe spot at 5.2. 1/50 with a faint trace of one.

Abdomen.—No scales at the postero-lateral angles of tergite 8; sometimes (8/25) with a few scales on sternite 6.

Adult Male.—Palps without basal pale band on segment III, median dorsal pale scales on segments II and III sometimes numerous. Wings sometimes (5/10) with a pale fringe spot at 5.2. A few slender pale scales on sternite 8 (6/6) and the usual ones on the coxites. *Terminalia* (fig. 6 d).—Phallosome with 4–5 pairs of leaflets, usually five, first two broad and serrate, the teeth sometimes large, fifth narrow and needlelike; small basal teeth may be present (*cf.* Russell and Baisas, 1936, Pl. VII, fig. 7b, especially the specimen from Mindanao which much resembles my fig. 6 d).

Larva.—Living larva bright green to brown without pale bands.

Head.—Inner anterior clypeal hairs simple, outer with 40–70 branches. Antennal shaft rather slender, the shaft hair commonly small, sometimes very small. Sutural hair with few branches, 6–9, average 7.

Thorax.—Mesothoracic hair 5 is distinctive (fig. 9 b) and at once distinguishes the larva of this species; the hair is small with 4–10 sinuate fine branches, spreading horizontally from the base.

Abdomen.—Palmate hairs a little smaller than those of *sinensis* but larger than those of *indiensis*, pigmentation uniform and fairly dense, extending a little into the tips. Segment V, hair 5, 4–7 branches, segment VI, hair 5, 5–8, hair 9, 5–9 branches. Tergal plate on VIII less transverse than in *sinensis*, three-quarters as long as wide, a little smaller, ratio length/width 0.65–0.82, average 0.73. Pecten with 7–9 long teeth, usually eight. Spiracles a little smaller than those of *sinensis* or *nigerrimus*, a little larger than those of *indiensis*.

Pupa (fig. 12 c).—Pigmentation light or moderate with the tips of the antennal case and bases of the paddles light; occasionally darkly pigmented. Wing cases with the usual pattern of lines and crossbars. Trumpet with some thickened and irregular areas on the rim. Hairs 2 and 5 with few branches, segment V, hair 2 with 2–6 branches, average about four, occasionally simple, hair 5 with 14–28, commonly

about 17 branches. Spine 8 with very much reduced branches or none at all. Paddle with stronger teeth than the foregoing species, extending much further, so that the refractile border is from 0.83 to 0.93 the length of the paddle, average 0.88.

Egg (fig. 13 *d, g*).—Resembles *nigerrimus* in the width of the deck, though a little wider, about a sixth as wide as the egg, but in side view the middle two-thirds of the deck is straight, not concave as in *nigerrimus*; also the floats are shorter with fewer ribs, only 25 to 30. Ends of the egg more pointed viewed from above.

For other illustrations of this species see *Adult* ♀, Baisas, 1931, Pl. I, figs. 5, 6; Russell & Baisas, 1936, Pl. VII; ♂ *terminalia*, Baisas, fig. 7 *b*, Christophers, 1933, ? fig. 25/5, 6; Russell & Baisas, 1936, Pl. VII; *larva*, Baisas, fig. 2 *d-g*, fig. 5 *d*; Russell & Baisas, 1934, Pl. 8; Baisas & Hu, 1936, Pls. III, IV; *pupa*, Baisas & Hu, Pl. V; Crawford, 1938 fig. 14; *egg*, Walch & Walch-Sorgdrager, 1935, Pl. III fig. 34; Baisas & Hu, Pl. 1; ? D'Abrera, 1944, fig. 4.

Identification.—Distinguished by the broad tarsal pale bands and a dark wing without pale scales on the base of the costa, or scales on the humeral cross vein, or fringe spot at 5.2. The anterior border of the remigium bears a well-marked line of white scales which contrast with the cream or orange pale scales on the rest of the wing. The pale scales on the base of 1 are usually well developed and extend back to the remigium. Compared with *nigerrimus* the propleural setae are fewer, 2-6, commonly four, and there are few pale scales on the mid coxa; scales absent from the tip of the abdomen. The larva is easily distinguished by the shape of mesothoracic hair 5; the antennae are rather slender, the shaft hair variable but often small, not nearly reaching to the end of the shaft, and the sutural hair has few branches, 6-9.

Notes.

Leicester described *peditaeniatus* from a large series bred from larvae collected from lakes and ponds around Kuala Lumpur where he found it a common species. The type specimen, together with other types of Leicester's, was lost in transit to Theobald at the British Museum (James & Stanton, 1912), and the specimens now in the collection there are cotypes selected by James and Stanton, from Leicester's collection. These cotypes are three females of the species described here as *peditaeniatus*, and two males of *indiensis*. The latter may safely be ignored, for Leicester's detailed description is mainly of the female and fits *peditaeniatus*, but not *indiensis*. In particular, he says that the wing scaling is much darker than that of *sinensis*, and this is also emphasised in his description of the latter. The wing fringe of *peditaeniatus* is described as all black except for the apical pale mark, but often with a pale spot at 5.2 in *sinensis*. He notices that the pale marks on the wings are inclined to be darker than in *sinensis*, more orange than cream or yellow.

Leicester's description of the larva has puzzled everyone, for he described the frontal hairs, which was the term then used for what we now call the clypeals (see Theobald, 1903, figs. 4, 24) as having only five or six fine branches on a long stem (unlike any form of "*hyrcanus*"), in contrast to those of *sinensis* which had numerous stiff bristle-like branches on a stout stem. Stanton (1915) who was familiar with the adult of *peditaeniatus*, and had helped to select cotypes from Leicester's material says "adult mosquitoes identical with Leicester's *peditaeniatus* are developed from larvae which do not differ from *sinensis* larvae". He suggests that Leicester may have been working with immature larvae, but this does not seem a convincing explanation. In 1930 Walch described and figured a larva found in a forest pool on Banka Island at a height of about 300 metres. It was similar to "*hyrcanus*" except that the outer anterior clypeal hairs had long stems and only a few branches thus agreeing with Leicester's description. Walch assumed it to be the larva of *peditaeniatus*. The following year Stoker (1931) found similar larvae in forest pools in Borneo, but on breeding out the adults they proved to be *A. montanus* Stanton & Hacker, 1917. He suggested that Leicester had mistakenly associated adults of *sinensis* and larvae of

montanus under the name *peditaeniatus*; this seems a possible though perhaps not a very likely explanation. Admittedly Leicester made many collections in jungle, but if he had found larvae of what was later called *montanus* he would probably have succeeded in breeding some adults. Even if he had failed in this and supposed the larvae to be related to *sinensis* he would probably have remarked on the unusual (jungle) breeding place. The chances of accidentally substituting the larvae of a rare jungle species for those of a common open country one differing from it seem rather remote. Gater (1933) also discusses the problem and assumes that Leicester must have described abnormal or immature specimens. One other explanation, perhaps no more likely than the others, occurs to me after reading the little that Leicester has to say about larvae. He seldom seems to have examined their chaetotaxy, for he bred many of the Anopheline species he describes from larvae, but except for remarks on *barbirostris*, *sinensis* and *peditaeniatus*, evoked largely by Theobald's notes on the first two (Theobald, 1903, p. 86*), he does not describe them at all, or only mentions the naked eye appearances. It is just possible, therefore, despite Theobald's drawings, that Leicester wrongly identified the "frontal" hairs. If one supposes that he is describing the sutural hairs and not the outer anterior clypeals, then a fair measure of agreement between his descriptions, and actual specimens is possible, as follows:— In comparing larvae of *sinensis* and *barbirostris*, he describes those of *sinensis* as having black heads and white bands; this strongly suggests *nigerrimus* as described here. *A. nigerrimus* has strong black sutural hairs with many branches (12–24), whilst *peditaeniatus* has few slender branches (6–9). The sutural hairs of *barbirostris* are also weaker and less branched than those of *nigerrimus*, but the outer anterior clypeals of the former have considerably stouter branches than *nigerrimus*. Leicester, however, describes the "frontals" of *sinensis* as much thicker than those of *barbirostris*.

Whatever the true explanation may be, it is clear that Leicester made some mistake in describing the larva of *peditaeniatus*; there is no doubt whatever about the identification of the three female cotypes. Baisas and Hu, and later Venhuis, described this species under the name *nigerrimus*.

On account of the distinctive mesothoracic hair 5 of the larva (fig. 9b) first described and illustrated by Russell and Baisas (1934), this species can be readily recognised from India to the Philippines. The adults can also be distinguished without much difficulty, but they do vary geographically. Specimens from Borneo, Siam, the Philippines and Indonesia are in general very similar to those from Malaya. Specimens from India and Assam differ somewhat; the rather characteristic pale scales at the base of vein 1 (usually silvery on the remigium) are very prominent, so that sometimes the whole vein from the base to the scale pale spot may be largely pale giving a distinctive appearance. Baisas and Hu found these pale scales at the base of vein 1 distinguished the wing of this species from other Philippine *hyrcanus* forms they examined. Specimens from Assam, and less frequently from India, also differ from Malayan ones in the narrowness of the tarsal pale bands. Sometimes the third hind tarsal pale band is much like that of *sinensis* and does not spread on to the base of the fourth segment. Very occasionally there may be a pale fringe spot at 5.2.

Distribution.

Ceylon, India from Bombay eastwards, Assam, Burma, Siam, Indo-China (Saigon), Malay Peninsula, Sumatra, Java, Borneo, Celebes and Philippines.

Type locality: Kuala Lumpur, Malay Peninsula.

Type: Lost, cotypes, 3 ♀, in Brit. Mus. Plesiotypes from Malaya with larval and pupal skins placed in Brit. Mus.

* Theobald's figure (1903 pp. 18), of the clypeal hairs of *sinensis* (*vanus*) and his description of the larva (pp. 86, 91) appear to refer to that of *A. annularis* V.d. Wulp which he had confused with *sinensis*.

Specimens seen.—INDIA : (B.M.), Marateru, 1919, 1 ♀ (Ramakrishna) ; Central Provinces, Sambalpur, 1 ♀ (Dr. Murphy), Bihar, Kiepur, ix.1915, 10 ♀ (India Museum) ; N. Kanara, Kavar, 1902, 6 ♀, 2 ♂ (Dr. H. Cogill) ; Bombay, Sholapur, 1903, 2 ♀ (Cogill). (S.), 2 ♀ 2 ♂.

CEYLON : (B.M.), Marlbe, xii.1922, 2 ♀, 2 ♂ (R. Senior-White) ; Saduganga, 1921, 2 ♀ (Senior-White) ; Peradeniya, 1900, 1 ♀ (E. E. Green) ; Dondra, 1907, 3 ♀ ; Colombo, 1914, 1 ♀ (K. McGahey). (L.S.H.T.M.), Kurunegala, 1913, 2 ♀. (S.), 1 ♀.

ASSAM : (L.S.H.T.M.), Shillong, 1921, 12 (McCombie Young) ; Jorhat Province, 28 (Dr. Hermette) ; Mangaldai, 1934, 5 ; Dhubri, 1934, 2 (Assam med. Res. Soc.) ; Juri, Sylhet, 1938, 2 ♀ (C. Hamilton). (A.), 1 ♀.

BURMA : (B. M.), 1925-27, 3 ♀, 1 ♂ (Maj. Bilberbeck) ; Lower Thaton, 1914, 1 ♀ (Fletcher). (L.S.H.T.M.), Shwenyaung, 1928, 1 ♀ (Feegrade) ; Myitkina, 1944, 3 ♀, Kabaw valley, 1944, 5 ♀, Kale Valley, 1944, 1 ♀ (? *peditaeniatus*, extreme base of costa pale, hind tarsal bands narrow) (T. T. Macan).

SIAM : (B.M.) Bangkok, x.1915, 2 ♀ (Dr. A. T. Stanton). (L.S.H.T.M.), 1920, 1 ♀ (M. E. Barnes). (S.), 6 ♀, Bangkok, 3 ♀.

INDO-CHINA : (P.), No. 25, 1 ♀ (Toumanoff) ; Saigon, 4 ♀, 3 ♂ (Borel).

MALAY PENINSULA : (B.M.), Perak, Taiping, 1899, 1 ♀ (L. Wray jun.) ; Selangor, Kuala Lumpur, 1903 and 1904, 3 ♀ (cotypes) (G. F. Leicester). (I.M.R.), numerous specimens have been seen from many localities, as far north as Kedah, Penang and Province Wellesley, and from Negri Sembilan in the south ; common around Kuala Lumpur.

SUMATRA : (B.M.), Fort de Kock, 1930, 3 ♀ (E. Jacobson). (B.), Fort de Kock, 19 ♀, Mandailing, 1 ♀, Kotta Radja, 2 ♀ ; Padang Sidempoean, 5 ♀. (A.), Fort de Kock, 1913, 2 ♀ (Jacobson), these and other specimens in this collection from here and elsewhere in Sumatra were labelled *peditaeniatus* det. Edwards.

JAVA : (B.), Tjilatjap, 5 ♀ 2 ♂ ; Tasik Malaya, 1 ♀ ; Kapetaken, 3 ♀ ; Batavia, 1 ♀. (L.S.H.T.M.), Buitenzorg, i.1930, 1 ♀ (R. W. Paine).

BORNEO : (I. M. R.), Labuan, viii-xii.1945, 10 ♀, iii.1948, 3 ♀, x.1948, 8 ♀ 6 ♂ (D. H. Colless) ; Jesselton, xi.1945, 1 ♀ (Colless).

CELEBES : (B.M.), Makassar, 1914, 1 ♀ (Dr. H. Werkman). (B.), Makassar, 4 ♀ ; Kalawara, 2 ♀ ; Kabaena, 1 ♀.

PHILIPPINES : (B.M.), 1906, 1 ♀ (Dr. G. F. Craig), 1910, 1 ♀, 1914, 2 ♀ (Miss Ludlow). (I.M.R.), Caluan, Laguna, ix. and x.1931, 2 ♀, 2 ♂ (W. V. King) ; La Mesa dam, Novalichos Rizal, 1 ♀ (F. E. Baisas).

Anopheles (A.) argyropus Swellengrebel, 1914.

Adult Female.—A very dark form with very broad white bands on the hind tarsi.

Head.—Proboscis dark. Palps with the first and second pale bands usually well developed, third and fourth bands may be confluent or partly so (29/50), median basal pale scales usually (46/50) present but pale scales between the bands generally absent. Instead the scales between the bands have a purple or lead grey sheen more pronounced than in *peditaeniatus*. This is readily visible also in living specimens in which the distal half of the palps is seen to be less shaggy than, for example, those of *indiensis*.

Thorax.—Pattern of the mesonotum much like that of *peditaeniatus*, but the chitin very dark, more dark scales ventro-laterally on the anterior promontories than in *sinensis*. Propleural setae 3-7, average 5.0.

Legs (fig. 3 g, h, j).—Very broad white bands on the hind tarsi, but comparatively narrow ones on the mid and fore tarsi. Coxal scales very few. Pale band at apex of hind tibia well marked and white. Fore tarsi ; bands similar to *sinensis* but whiter, second band as usual the longest 3.3–4.4 times as long as wide, average 3.5 about 1/3 as long as the segment, occasionally (7/50) extending across the joint. Mid tarsi ; second band 1.4–1.8 times as long as wide, average 1.7, third band is usually the longest (17/20) and varies from 0.2 to 0.3 times the length of segment III, being usually 1/4 or less. Hind tarsi ; four bands, of which the second to fourth always extend on to the next segment and the first does not, the third band is the longest, and in some specimens may fuse with the fourth, which may extend to the tip of the fifth segment so that the hind leg is continuously white from the tip to half way up the third tarsal segment, although in such specimens there are often a few scattered dark scales on the fifth and fourth segments. In less broadly banded specimens in which the third band does not fuse with the fourth, the former is from 8.0–8.9 times as long as wide which corresponds to 1.6–1.9 times the fifth segment, or 0.8–0.9 times the fourth.

Wings (fig. 2 c).—Resembling those of *peditaeniatus* in several respects, such as the absence of any pale scales on the basal half of the costa or a fringe spot at 5.2, but darker with reduced pale areas on costa and vein 1. Length, 3.14–3.94, average 3.46 mm. Differs from *peditaeniatus* chiefly as follows : Humeral cross vein with a tuft of dark scales (50/50) ; anterior border of the remigium with fewer pale scales, and those not always very white, scales mainly dark in about half the specimens. Pale marks on the costa and vein 1, usually cream to ochreous. Subcostal pale area small, often reduced to a small fleck of ochreous scales, occasionally (2/50) absent, usually (47/50) not present at all on vein 1, though forming a minute pale tip to the subcosta. Vein 1 ; scattered pale scales between remigium and sector pale area much fewer, varying from 0 (3/50) to about 20 (3/50), sector pale area sometimes reduced to only 2–3 scales, pale scales on the middle dark mark commonly about ten, varying from 0 (1/50) to about 25, no pale scales between subcostal pale area and preapical pale spot (50/50), the latter is of moderate to small size. Apical fringe spot variable and sometimes considerably reduced, commonly extending from vein 1 (44/50) to vein 3 (32/50), the widest extend from above vein 1 (4/50) to beyond 3 (11/50), and the narrowest from below 1 (2/50) to 2.2 (2/50). Stem of vein 2 entirely dark. The scattered dark scales on vein 3 between basal and apical dark marks are mainly in the distal half. Vein 5 with dark scales at extreme base as usual, the pale area shorter than the succeeding dark mark, occasionally (3/50) absent, dark mark about the same length as in *peditaeniatus*, 0.3–0.5 mm. but less often (1/50) ending level with the upper dark mark on 6. Apical dark mark on 5.2 commonly (33/50) equal to or longer than that on 6, in *peditaeniatus* always shorter (50/50).

Abdomen.—Very dark, seldom (3/26) with any scales at the postero-lateral angles of tergite VIII, 1/26 with some pale scales on sternite VIII.

Adult ♂.—Palps without a basal pale band on segment III, but often with a pale mark towards the apex of II. Usually no scales on sternite VIII, but pale scales on the coxites. *Terminalia* (figs. 5 f, 6 e).—Only two pairs of leaflets, both serrate, basal teeth present.

Larva.—Usually dark brown without pale bands.

Head (fig. 7 b).—Inner anterior clypeal hairs simple, outer much as in *nigerrimus*, perhaps a little less dense and stiff. Sutural hairs as in *nigerrimus* with many branches, 12–22, average 17. Spines on the inner edge of the antennal shaft usually rather coarse and erect, the shaft slender compared with *nigerrimus*, the shaft hair usually reaching to or beyond the end of the shaft.

Thorax.—Mesothoracic hair 5 with 6–12 branches.

Abdomen (figs. 7 h, 10 a).—Palmate hairs similar to those of *peditaeniatus* in size and pigmentation. Segment V, hair 5, 4-5 branches, segment VI, hair 5, 5-6, hair 9, 4-5 branches. Tergal plate on VIII with very similar dimensions to those of *peditaeniatus*, three-quarters as long as wide, ratio length/width 0.67-0.80, average 0.73; the shape is commonly that of a truncated wedge (fig. 7h). Pecten with 6-8 long teeth, commonly seven. Saddle hair strong, longer than the width of segment VIII, or the length of the saddle.

Pupa (fig. 11 d).—A very dark form with pigment well developed around the bases of most of the hairs, in addition to the base of the paddles, ♂ genital plate, wing and antennal cases, etc. Trumpet distinctive, with a plicate appearance due to somewhat irregular vertical grooves or folds. Hairs 2 and 5 with many branches forming dark tufts; segment V, hair 2, with 17-40 branches, hair 5 a bushy dark tuft. Spine VIII and paddle teeth similar to *peditaeniatus*, the former with reduced branches, the latter strong and extending far down, refractile border 0.81-0.91, average 0.87 the length of the paddle.

Egg (fig. 13 h, i).—Immediately distinguished from the eggs of the foregoing species by the extremely narrow deck, less than one-twentieth as wide as the egg, and the prominent points. The deck is slightly convex in side view as in *indiensis*, and this, with the narrower deck, partly accounts for the prominence of the points, so that when viewed from above the ends of the egg are not rounded as in *sinensis*, but narrowed to the projecting bulbous points. The bosses on the points (commonly four) are large and white, appearing to be covered by an extension of the frill membrane; the bosses of *sinensis* appear small, bare, and slightly shining black by comparison. Floats with about 30-35 ribs.

For other illustrations of this species see *Adult* ♀, Swellengrebel, 1921, Pl. XVI; Swellengrebel and Rodenwaldt, 1932, Pl. IV, fig. 4; Gater, 1935, fig. 123 b.

Identification.—Easily distinguished as a rule by the very broad white hind tarsal bands, combined with narrow ones on the mid tarsi. The wing is usually very dark with few pale scales on vein 1 (none between Sc and preapical pale spots) and the subcostal pale spot often narrow and occasionally absent. Dark scales on the humeral cross vein, no fringe spot at 5.2. Dark scales of the palps often with a dull purple sheen. The larva is usually darkly pigmented and is distinguished by the characters in the key.

Notes.—Swellengrebel (1914) described specimens of "*hyrcanus*" with very broad pale bands on the hind tarsi as variety *argyropus*. The width of the hind tarsal pale bands alone is not sufficient to distinguish all specimens, for the species overlaps in this character with *peditaeniatus*, but it is clear from the description that Swellengrebel had true *argyropus* before him. He describes the hind tarsus as white from the distal half of segment III to the apex of V, but with a trace of brown scales in the middle of IV; this is very typical of the broadest banded specimens. Furthermore, the fore tarsi are shown as having narrow pale bands, and the basal dark mark on vein 5 is very long.

Specimens from Assam show the same tendency to shortening of the tarsal pale bands as occurs in *peditaeniatus*, and the hind tarsal bands may be no broader than those frequently seen in *peditaeniatus* from Malaya. However, the distinction between the two species on the relative width of the mid and hind tarsal bands seems to hold good.

Distribution.

Specimens have been seen from India, Assam, Siam, Malay Peninsula and Java, and it presumably occurs also in Burma and Sumatra. Brug and Bonne-Wepster (1947) give also Alor, Timor and Ternate, but this requires checking as confusion with broad tarsal banded *peditaeniatus* is possible.

Type locality : Deli, Sumatra.

Type : None. Plesiotypes from Malaya with larval and pupal skins placed in Brit. Mus.

Specimens seen.—

INDIA : (S.), ? loc. 7 ♀.

ASSAM : (L.S.H.T.M.), Jorhat Province, 4 (Dr. Hermette) ; Mangaldai, 1934, 2 (Assam med. Res. Soc.).

SIAM : (B.M.), Bangkok, x.1915, 2 ♀ (Dr. A. T. Stanton). (S.), Bangkok, 2 ♀.

MALAY PENINSULA : (I.M.R.) Province Wellesley, 1 ♀ ; Perak, Bagan Datoh, Melintang estate, xii.1940, 1 ♀ ; Kuala Lumpur, Kenny road, xii.1947, 1 ♀, Ampang road, xi.1946, 1 ♀ ; 5th mile, Klang road, 1950, many specimens ; Negri Sembilan, Ulu Jempol, xii.1940, 1 ♀ ; Pahang, Kuantan-Pekan road, 1941 and 1950, many specimens.

JAVA : (B.), Batavia, 3 ♀, Cheribon, 4 ♀. (S.), xi.1933, 2 ♀ (R. Soesilo).

Anopheles (A.) lesteri Baisas & Hu, 1936.

Subspecies near *sinensis* of Colless, 1948.

Adult Female.—A moderately dark form, general appearance of the wings somewhat like those of *peditaeniatus*, but having a very short apical fringe spot (in Malayan and Bornean specimens) and *sinensis* type hind tarsal bands.

Head.—Proboscis, mean length in five specimens 1.95 mm. ratio fore femur/proboscis 0.95. Bands of the palps usually distinct, first and second always present, third and fourth seldom (6/50) confluent, median basal pale scales present in less than half (22/50), scattered pale scales between the bands rarely present (2/50). The distal two-thirds of the palps are somewhat less shaggy than in other forms, and thus appear a little thinner, especially in the apical one-third.

Thorax.—Appearance of the mesonotum very similar to *sinensis*. Propleural setae 3-6, average 3.9.

Legs (fig. 3 c).—Rather dark, pale bands at base and apex of hind tibia not well developed. Coxal scales few or absent. Fore tarsal bands narrower than in *sinensis*, range of variation rather small, first and second nearly equal, second usually (16/20) slightly the longest, a minute fourth band present in about half (10/20), but signs of basal bands rare (2/20), second band 1.7-2.6 times as long as wide, average 1.9, corresponding to about one-fifth or less the length of the segment. Mid tarsal bands similar to, but shorter than those of the fore tarsi, second band 1.5-1.9 times as long as wide, average 1.7. Hind tarsi ; bands 1, 2 and 3 about equal, 4 always present but slightly shorter, occasionally (8/20) extending on to the fifth segment, third band occasionally extends very slightly on to the base of the fourth segment, slightly wider than in *sinensis*, range of variation rather small, from 1.4-2.9 times as long as wide, average 1.7.

Wings (fig. 1 c).—Length 3.40-4.33, average 3.82 mm. The main features are as follows. Costa without any pale scales between base and subcostal pale area. Humeral cross vein usually bare, occasionally (5/50) with one or two dark scales. Remigium with light and dark scales, but usually (40/50) those of the anterior border are mainly dark. Vein 1 between remigium and sector pale area usually (47/50) with some scattered pale scales, commonly about 10-20, up to about one-third to one-half the scales pale. Sector pale area always present but sometimes small and incomplete. Pale area on the middle dark mark variable, sometimes well developed and fully connected with the subcostal pale area, sometimes reduced or absent. Subcostal pale area always present, but on the average a little narrower than in *sinensis*,

and seldom (4/50) complete on vein 1. Vein 1 between subcostal and preapical pale area often (25/50) without any pale scales, when present maximum is about 20. Preapical pale area narrower on the costa than in *sinensis*. Apical fringe spot very narrow, maximum extent usually from opposite or occasionally above the termination of vein 1 (26/50), to vein 2.2 or occasionally a little beyond (16/50), commonly from below vein 1 (24/50) to above 2.2 (34/50). Vein 5 usually with a few dark scales at extreme base followed by a more or less well defined pale area, distal to which is the usual dark mark, which is fairly long, 0.3–0.5 mm., usually (31/50) separated by less than its own length from the upper dark mark on 6, or by its own length (17/50). Vein 6; upper dark mark shorter than or equal to the apical one (46/50), apical mark always longer than the apical one on 5.2. No pale fringe spot at termination of 5.2 (50/50).

Abdomen.—No scales at the tip (postero-lateral angles of tergite VIII). Ventral tuft on sternite VII tends to be rather small.

Adult Male.—Palps without a basal pale band on segment 3, the usual median dorsal pale scales present along the length of segments 2 and 3. No pale scales on the dorsal surface of the genital coxites, only setae. *Terminalia* (fig. 6*f*).—Phallosome with four pairs of leaflets of which the first two bear teeth. Basal teeth present.

Larva.—Resembles that of the next species, *crawfordi*; usually dark brown, opaque, with a blackish head and brown antennae.

Head (figs. 7*d*, 8*d*).—Inner anterior clypeal hairs simple, outers with about 50–70 short, stiff black branches. Sutural hairs, with 5–11, average 9 branches. Antennal shaft hair usually reaching to or beyond the end of the shaft.

Thorax.—Inner shoulder hairs with 2 or 3 small branches near the tips. Meso-thoracic hair 5 with 3–6 straight branches.

Abdomen (figs. 7*f*, 8*b*, 9*a*).—Palmate hairs similar to those of *crawfordi*, though slightly larger. Segment II, hair 5, 6–10 branches, average 9; segment V, hair 5, 4–6; segment VI, hair 5, 5–8, hair 9, 4–7 branches. Tergal plate on VIII similar to that of *crawfordi*, but somewhat larger, ratio length/width 0.59–0.82, average 0.72. Pecten with 7–10 long teeth, usually 8. Saddle hair not weak, about as long as the width of segment VIII. Anal papillae usually very much shorter than those of the other forms, less than the length of the saddle or the width of segment VIII, this character, however, is probably governed by the salinity of the breeding place.

Pupa (fig. 12*e*, *f*).—Darkly pigmented, very similar to *crawfordi* in most respects including the thickened rough areas on the rim of the trumpet. Hair 2 on segment VI with more branches, 5–10, but hair 5 on segment V with fewer branches, 12–20; hair 2 on V about the same, 7–13 branches. Refractile border of paddle very short, 0.52–0.64, average 0.58 the length of the paddle.

Egg (fig. 13).—Similar to those of *argyropus* and *crawfordi*, but with a slightly wider deck about one-tenth or less as wide as the egg. Bosses on the points small. Floats with about 24–28 ribs.

For other illustrations of this species see *Adult* ♀, Russell and Baisas, 1936, ? Pl. 8, fig. 4; Colless, 1948, figs. 6*a*, *b*; ♂ *terminalia*, Russell and Baisas, 1936, pl. 8; Colless, figs. 6*c*, *d*, *e*, ; *larva*, Russell and Baisas, 1934 ? Pl. 9, figs. 1, 2; Baisas and Hu, 1936, Pls. 3 and 4; Colless, figs. 6*g*, *h*; *pupa*, Baisas and Hu, Pl. 5; *egg*, Baisas and Hu, Pl. 1.

Identification.—Distinguished by the combination of narrow apical pale bands on the hind tarsi not extending across the joints, rather narrow dark wing with very narrow apical fringe spot (not narrow in Philippine specimens), long dark mark on the base of 5, no scales on the humeral cross vein, no fringe spot at 5.2, palps rather slender distally with narrow well marked pale bands and few scattered pale scales, no scales on the mid coxae, and only 3–6, commonly 4, propleural setae.

The male lacks pale scales on the apparent dorsal surface of the coxites, a character peculiar to this species. The larva is usually darkly pigmented with a rather dark antenna, the shaft hair usually reaching beyond the end of the shaft, short black outer clypeal hairs, small densely pigmented palmate hairs, and short anal papillae. The most usual type of breeding place is near or in slightly brackish water, and this probably accounts for these papillae being short, and in combination with the characters listed, makes identification fairly easy.

Notes.

Dr. Rozeboom very kindly loaned me a large amount of material of '*hyrcanus*' which he had collected and reared on Samar and Luzon in the Philippines. This proved to be nearly all *lesteri*, and examination has shown that the Malayan form we had been calling the 'coastal form' is very closely similar. The only obvious difference is that the Philippine specimens have a normal apical fringe spot, instead of the characteristic narrow one found in Malayan and Bornean specimens. Otherwise, with the possible exception of the eggs, no important difference has been noted. Philippine *lesteri* have all the other marks that characterise this form in Malaya e.g., terminal third of the female palps rather thin, no pale scales on the mid coxae, few (3-5) propleural setae, rarely any scales on the humeral cross vein (Baisas and Hu say these are present); no fringe spot at 5.2, no pale scales on the coxites of the male genitalia, phallosome with leaflets exactly like those illustrated (fig. 6f), except that there commonly seem to be five pairs instead of four. The larval and pupal chaetotaxy of Malayan and Philippine specimens seems to be almost identical (Table V), and both have pupal trumpets with thickenings on the rim (fig. 12e, f). The eggs of Philippine *lesteri* have rather wider decks than the Malayan, and rather more ribs on the floats. It seems therefore, that though specimens from Malaya and Borneo are not quite identical with typical *lesteri* from the Philippines, the differences are not of more than subspecific rank, and it hardly seems necessary at present to distinguish them by different names, especially as there is no question as yet of creating subspecific names for the geographical variants of other forms such as *peditaeniatus*, though admittedly these are perhaps less clear cut. The subspecies near *sinensis* described from Borneo by Colless (1948) is clearly this species.

Distribution.—Only known so far from the Malay Peninsula, Borneo and the Philippines.

Type locality: Santa Mesa, Manila, Philippines.

Type: ♂ and ♀ with corresponding larval and pupal skins in Philippine Nat. Mus. Cotypes in U.S. Nat. Mus., Washington, and Henry Lester Inst. Med. Res. Shanghai. Plesiotypes from Malaya with larval and pupal skins placed in Brit. Mus.

Specimens seen.

MALAY PENINSULA: (I.M.R.), many specimens from localities on the west coast, from Kedah, Penang, Province Wellesley, and Perak, to Selangor; also from Kuantan on the east coast, and from Johore and Singapore. From inland localities, 3 ♀ from Kuala Lumpur 1948-50.

BORNEO: (I.M.R.), Kuala Belait, viii.1945, 1 ♀, v.1948, 1 ♀, 2 ♂; Labuan, iii.1948, 3 ♀, 1 ♂; Brunei, vii, viii.1945, 3 ♀ (D. H. Colless).

PHILIPPINES: Samar and Luzon, 1945, many specimens (Dr. L. E. Rozeboom).

Anopheles (A.) crawfordi, sp. n.

A. hyrcanus pupal type B, Crawford, 1938.

Adult Female.—With a sharp bright wing pattern, and well marked eye spots on the mesonotum as in *indiensis*, but the *sinensis* type of hind tarsal bands.

Head.—Palps ; bands 1 and 2 nearly always present, 3 and 4 usually (45/50) separate, basal median pale scales sometimes (15/50) absent, scattered pale scales between the bands usually (36/50) absent.

Thorax.—Mesonotum as in *indiensis*. Propleural setae, 2-5 average 2.9, sometimes (5/10) with one or two scales on the lower mesepimeron.

Legs (fig. 3 b).—Coxal pale scales scanty, upper mid coxal setae, 3-4, average 3.6. Fore tarsi ; bands 1 and 2 equal or 1 longest, (42/50), seldom (5/50) any fourth band, and no basal bands, second band 1.8-4.1 times as long as wide, average 2.8, about 1/4 or less the length of segment 2. Mid tarsi ; pale bands similar to those of the fore tarsi but shorter. Hind tarsi ; the bands are generally slightly longer than in *sinensis*, bands 1, 2 and 3 are of about equal length, 4 is always present and often nearly equal to the others, rarely (2/20) the third or fourth bands may extend very slightly across the joints, third band from 1.1-3.1 times as long as wide, average 2.0.

Wings (fig. 1 d).—Length 2.94-4.20, average 3.78 mm. Much resembling *indiensis* in general pattern, differing as follows. Costa between base and subcostal pale spot without pale scales, occasionally (7/50) there may be 2 or 3 minute ochreous scales in the region of the humeral cross vein, and in one specimen these formed a small ill-defined humeral interruption. Humeral cross vein bare (24/50), or with one or two dark scales (26/50), but not a tuft. Subcostal pale area usually (43/50) complete on vein 1 which lacks (50/50) any pale scales between this and the preapical pale area. The preapical pale area appears to have shifted distally compared with *sinensis* so that the tip of vein 1 is nearly always pale (47/50), and the apical fringe spot commences lower down and is narrower in consequence, it usually commences at 2.1 (30/50) and ends only slightly beyond 3 (42/50), or at 3. Vein 5 ; basal dark mark short, much as in *indiensis*, 0.1-0.3 mm. long, separated by up to 3 times its length from the upper dark mark on 6. Vein 6 ; upper dark mark usually equal to or longer than the apical (40/50), which is very seldom (2/50) longer than the apical dark mark on 5.2. No pale fringe spot at 5.2 (49/50).

Abdomen.—No scales at the postero-lateral angles of tergite VIII (24/25).

Adult Male.—Palps without, or with only a small pale band at the base of segment 3. Wings often (7/8) with a fringe spot at 5.2 occasionally (2/8) with one or two pale scales on the costa near the humeral cross vein. Apical fringe spot rather narrow as in the female. No pale scales on sternite VIII, and few on the coxites. *Terminalia* (figs. 5 g, 6 g). Phallosome with 4-6 pairs of leaflets, usually 5, the first pair bears teeth, basal teeth present.

Larva.—Black to yellowish green or green, usually opaque dark brown. Without pale transverse bands, but there may be a dull longitudinal pale line on the abdomen, and a dull pale mark on the thorax.

Head.—Inner anterior clypeal hairs simple, outer with about 50 branches. Suture hairs 6-10 average 9 branches. Antennal shaft hair variable, more often longer than the shaft than shorter. *Thorax*.—Inner shoulder hair simple, or with up to 8 small branches, occasionally this hair is indistinguishable from that of *A. barbirostris* with long branches from the base. Mesothoracic 5 with 5-8 stiff branches. *Abdomen*.—Palmate hairs small, usually with dense black uniform pigmentation, ending abruptly before the leaflets narrow to the tips. Segment II, hair 5, 10-18 branches, average 13 ; segment V, hair 5, 5-9 branches ; segment VI, hair 5, 6-10, hair 9, 5-8 branches. Tergal plate on VIII small, shape rather variable but nearly always between two-thirds and three-quarters as long as wide, ratio length/width 0.59-0.81, average 0.71. Pecten with 7 or 8 long teeth. Saddle hair weak, much as in *indiensis*, usually shorter than the width of segment VIII. Spiracles small.

Pupa.—Dark, with tips of antennal cases and bases of paddles pigmented. Trumpet with thickened or rough areas on the rim. Segment VI, hair 2 with 2–4 branches; segment V, hair 2, 7–19 branches, hair 5 a tuft of 30–40 branches. Refractile border of paddle 0.60–0.70, average 0.65 the length of the paddle.

Egg (fig. 13 k).—Closely resembles that of *argyropus*, having a very narrow deck, slightly convex in profile. Differs only slightly, the points are not quite so prominent nor the bosses so large, and the float ribs are rather fewer, about 27–30. Occasionally the deck may be interrupted once or twice so that it appears as two or three complete but disconnected lengths.

For other illustrations of this species see Crawford (1938), fig. 11 (pupa).

Identification.—Characterised by the *sinensis* type of hind tarsal bands (narrow and not extending across the joints), with a sharp bright wing pattern and mesonotal eye spots like *indiensis*. Characters of the wing not mentioned in the key are the lack of pale scales on the basal half of the costa, few or no scales on the humeral cross vein, and the rather narrow shape of the wing like that of *lesteri*, contrasting with the broader wings of *sinensis* and *nigerrimus*. There are few propleural setae, 2–5, usually 3, and few pale scales on the mid coxae. The larvae are usually dark and are very similar to those of *lesteri*, but may be distinguished as in the key. Sometimes prothoracic hair 4 tends to have branches rather crowded towards the tip in a manner reminiscent of the *umbrosus* group.

Notes.—This is Crawford's pupal type B. He kindly presented to this Institute two adult males hatched from his pupae.

Distribution.—So far recognised only from the Malay Peninsula and Sumatra.

Type locality: Kuala Lumpur, Malay Peninsula.

Type: Holotype ♀ and allotype ♂ with their larval and pupal skins, plus paratypes, placed in Brit. Mus.

Specimens seen.

MALAY PENINSULA: (B.M.), Taiping, 1899, 3 ♀ (L. Wray). (I.M.R.), Perak, Tanjong Malim, 1941, 1 ♀; Selangor, Kuala Kubu Bahru, 1940, 1941, many ♀, Sungei Choh, 1941, 1 ♀; Kuala Lumpur district, Sungei Tua, 1940, 1 ♀, Ulu Klang, 1940, 1950, several ♀, Kepong road, 1950, many ♀, Sentul, Kenny road, 5th mile Klang road, etc., 1931–1950, a number of specimens, Seafeld estate, 1940, 6 ♀; Negri Sembilan, Ulu Jempol, 1941, 1 ♀; ? Johore, Muar, 2 ♂ (R. Crawford); Pahang, Bentong, 1941, many.

SUMATRA: (B) Rao, 1 ♀.

Species D2, near *nigerrimus*.

This form, which appears to be yet another species, has only recently been detected amongst material from the north of Malaya where it has been collected with *sinensis* and *nigerrimus*, and there has been insufficient time or material to make a thorough study. It was at first mistaken for a variation of *lesteri* since it has a narrow apical fringe spot like that species, but further examination, especially of the larvae, pupae and phallosome leaflets, shows that it is rather closely related to *nigerrimus*. Since *nigerrimus* has been known to us here for some time as D, from Crawford's designation of the pupa, D2 is a convenient label. It appears to be a small species with a wing length of little more than 3 mm. The wing pattern is much like that of *sinensis* with a rather short basal dark mark on 5, but a narrow apical fringe spot. There are dark scales on the humeral cross vein and pale scales on the remigium, and in many males and a few females there are a few pale scales on the costa tending to form a humeral pale interruption. Some males show a faint pale fringe spot at 5.2. Propleural setae are about 2–4, the coxae bear pale scales, and the hind tarsi have the *sinensis* type of banding restricted to the apices of the segments. The male palps have a pale band on the base of the third segment

as in *nigerrimus*, the coxites bear pale scales, and the phallosome has only two pairs of leaflets, the larger of which seems to have teeth on both edges as shown for *nigerrimus* (fig. 6 b). The smaller pair of leaflets appears to be plain, and narrower than those of *nigerrimus*. Examination of some of the larval hairs, particularly those which are of value in identifying *nigerrimus*, reveals very little difference between the two, and at present the larvae could not be distinguished. The pupae have numerous branches on abdominal hair 5, though perhaps the hair is not quite as strong as in *nigerrimus*; the appearance of the trumpets and general pigmentation is very similar, but D2 has 2-5 branches on the terminal paddle hair against 1-2 for *nigerrimus*. In this respect, and in some of the larval hair counts it resembles *williamsoni*, but the latter has 7-12 branches on mesothoracic hair 5 against 4-7 for D2.

In the key to adults, D2 would run to *lesteri*, but could be distinguished by the scales on the humeral cross vein, short basal dark mark on 5, and pale scales on the mid coxae.

Specimens seen.

MALAY PENINSULA: (I.M.R.), Kedah, Kg. Ayer Hitam, 8.ii.1949, larvae in *Pistia* covered ponds with *A. ramsayi* and a few *nigerrimus* (see Reid, 1950) 7 ♀ 7♂, Kedah, Bukit Meriam, 12. ii. 1951, night catch, 3 ♀.

Before leaving the descriptions, it may help to form a picture of these species if some of the more distinctive characters of each are summarised in general terms, drawing on all stages of the life-history. These characters are more precisely and fully defined in the keys and descriptions.

A. sinensis.—Hind tarsal pale bands narrow, apical only. Wing with a short dark mark at the base of vein 5 and often a pale fringe spot at termination of 5.2. Egg with a broad deck one-third its width. Pupal trumpet with a thin uniform rim, a pattern of round dark spots on the wing case, and rather few branches on hair 5. Larva commonly green with rather large slender-pointed palmate hairs.

A. nigerrimus.—Third hind tarsal band extends a little across the joint on to the base of segment IV. Dark mark on the base of vein 5 long, often one or two pale scales on the basal third of the costa and a fringe spot at 5.2. Male palps with a pale band on the base of the third segment. Larva with many branches on the sutural hair and few on abdominal hairs 5 and 9. Pupal trumpet with a thin uniform rim, hair 5 with many branches forming a dark tuft.

A. indiensis.—Hind tarsal bands fairly broad extending across the joints. Wing with a bright sharp pattern and scattered pale scales on the basal third of the costa, basal dark mark on 5 short, often a fringe spot at 5.2. Egg with a fairly broad deck divided into two areas at either end.

A. peditaeniatus.—Hind tarsal bands broad, mid tarsal bands fairly broad. Wings with white scales on the remigium and cream scales on the base of vein 1, no pale scales on the base of the costa, humeral cross vein bare, no fringe spot at 5.2 (except some males). Larva with mesothoracic hair 5 of distinctive shape (fig. 9 b), sutural hair small with few branches. Pupal spine eight without branches, paddle with a long refractile border, hairs 2 and 5 on the abdomen with few branches.

A. argyropus.—Hind tarsal bands very broad, mid tarsal bands narrow. Wing dark with no pale scales on vein 1 between subcostal and preapical pale spots, and no fringe spot at 5.2. Pupal spine eight and refractile border of paddle as in *peditaeniatus*, but trumpet with vertical folds, and hairs 2 and 5 with many branches forming tufts. Egg with a very narrow deck less than one-twentieth its width.

A. lesteri.—Hind tarsal bands narrow, apical only. Wing with a very narrow apical fringe spot (except Philippine specimens), humeral cross vein bare, dark mark

on the base of 5 long and no fringe spot at 5.2. Genital coxites of the male without pale scales. Pupal trumpet with thickened coarsely toothed areas on the rim, refractile borders of paddle short.

A. crawfordi.—Hind tarsal bands narrow, apical only. Wing with a bright sharp pattern and short dark mark on the base of 5 as in *indiensis*, but (except for some males) without pale scales on the base of the costa or fringe spot at 5.2. Vein 1 with the tip pale and no pale scales between subcostal and preapical pale spots. Pupal trumpet as in *lesteri*. Eggs with a very narrow deck as in *argyropus*.

Classification.

Status as species.

Although cross-mating experiments have not been made, it is quite clear that the named forms described here are true species. They can be distinguished in all stages of the life-history (including small differences in the number and shape of the leaflets on the male genitalia); they occur together in the same areas (sympatric), and often larvae of two or three may be found within a few yards of one another in the same breeding place, no intermediate specimens have been encountered in Malaya, and offspring of one parent are of the same form as the parent (see Tables I & II). There is evidence of considerable biological differences between them, although information so far is fragmentary.

TABLE I.
Progeny raised from eggs laid by single wild-caught females. 1950.

Parents*		Progeny	
Species and Number		Number and Species	
<i>sinensis</i>	3	18	<i>sinensis</i>
<i>nigerrimus</i>	2	27	<i>nigerrimus</i>
<i>indiensis</i>	1	11	<i>indiensis</i>
<i>peditaeniatus</i>	2	11	<i>peditaeniatus</i>
<i>argyropus</i>	1	21	<i>argyropus</i>
<i>crawfordi</i>	2	2	<i>crawfordi</i>
Total	11	90 (44 ♀, 46 ♂)	

* Eggs were obtained from *lesteri*, but no adults were reared.

Lamborn (1922a) at Kuala Lumpur made a long series of progeny rearings of a number of species, including the *hyrcanus* group, and his specimens have been examined in the Hope Museum at Oxford. Unfortunately some of his series have been mixed up; for example, a long series of *crawfordi* ostensibly reared from a single female, contains one *A. barbirostris* female and many *sinensis*. Nevertheless there are a number of uncontaminated series, and in some of these the tendency for familial resemblance in minor details between the offspring and the parent is well shown. The uncontaminated series seen were as in Table II, page 46.

These forms then are distinct though very closely related species. They appear to be good examples of what Mayr means by *sibling* species; he says (Mayr, 1947, p. 150) "... several species which occur at the same locality, are so similar (sibling species) that they had been considered individual variants of one species". Unlike the *Anopheles umbrosus* group (Reid & Hodgkin, 1950) in which there are important structural differences between the species, even though they may be superficially very similar, these species of the *hyrcanus* group can be distinguished only by a combination of several, mostly small, characters. Even so, single adult specimens of either sex (and usually larvae and pupae also) can always be identified.

TABLE II.

Progeny reared from single wild-caught females. Lamborn's specimens from Kuala Lumpur.

Parents. Species and number		Number of ♀ Progeny
<i>sinensis</i>	4	98
<i>nigerrimus</i>	1	28
<i>indiensis</i>	2	26
<i>crawfordi</i>	3	20
Total	10	172

Nomenclature.

If we accept these forms as species, we have to consider what effect this will have on the nomenclature now in daily use. Evidently the names *nigerrimus* and *sinensis* can no longer be used to denote merely forms with broad or narrow hind tarsal bands, but must be restricted to the species described here under those names. Ideally all specimens should be identified to the species, but it is certain that this will not be done in practice. Routine malaria control is concerned more often with larval than adult mosquitos, and though the experienced worker should find no difficulty in identifying the adults of these species if they are not too badly damaged, and he does not rely solely on a hand lens, the identification of some of the larvae would be laborious and not worth the trouble, since the species are not very often vectors of disease. A possible solution seems to be to retain the name *hyrcanus* for the group as a whole, or unidentified members of it, but to indicate that it is used in this sense, and not as meaning Pallas' type form from the Caspian, by writing it in inverted commas, '*hyrcanus*', as was done with the *umbrosus* group (Reid & Hodgkin, p. 315 footnote). This method has already been used by Mayr (1947), and doubtless by others. Mayr (p. 200) writes 'Several such incompletely analysed species groups occur in the genus *Drosophila*. *Drosophila* "*obscura*" and "*affinis*" are best known,'. Whether one should use single or double inverted commas is probably a matter of choice (Fowler, 1949); single inverted commas are quicker to write manually, and take up less space in a typescript table. *Anopheles* '*hyrcanus*' will then be a shorter way of writing *Anopheles hyrcanus* sibling species group, the specific name in inverted commas indicating a sibling species group instead of a single species. Some workers would perhaps prefer the term superspecies as an alternative to sibling species group, but Mayr seems to be right when he restricts the use of superspecies to describe those cases where the geographically separated units of a polytypic species have differentiated so much that they seem more likely to be species than subspecies. The superspecies is then the highest category that can be claimed to have an objective existence. The sibling species group on the other hand is sympatric by definition, and in some instances probably represents the next evolutionary step when the members of a former superspecies have come to overlap widely.

Some uses of the inverted comma method will be apparent in the succeeding pages where '*hyrcanus*' is employed to refer to the group of species, and, as in the paper on the *A. umbrosus* group, to discuss earlier records where the exact identification is in doubt. It should also be useful to the Health Officer and his staff concerned with the day to day work of malaria control, who wish to be correct in their use of names, but who could not justify the time and trouble necessary for the exact

identification of these species, at least in the larval stage, unless there was cause to suspect that they were concerned in disease transmission. The same situation has arisen in the *Anopheles leucosphyrus* group (Reid, 1949), now being studied in detail by Colless, and it may arise in other groups such as *A. barbirostris*. Most of the members of the *A. umbrosus* group should not present any great difficulty in identification, and as a rule it ought not to be necessary to resort to this device with these species, but it may be useful to those who have to work with *A. 'maculipennis.'*

Synonymy.

The oriental forms of '*hyrcanus*' recognised by Christophers in 1933, with their synonyms, are compared below with those recognised in the present paper.

Christophers, 1933	Present paper
1. <i>Anopheles hyrcanus</i> Pallas, 1771. var. <i>sinensis</i> Weidemann, 1828. syn. <i>plumiger</i> Dönitz, 1901. <i>jessoensis</i> Tsuzuki, 1901. var. <i>nigerrimus</i> Giles, 1900. syn. ? <i>nero</i> Doleschall, 1851. <i>indiensis</i> Theobald, 1901. ? <i>pursati</i> Laveran, 1902. <i>bentleyi</i> Bentley, 1902. <i>minutus</i> Theobald, 1903. <i>argyropus</i> Swellengrebel, 1914.	<i>A. hyrcanus</i> species group. — 1. <i>A. sinensis</i> Wied. syn. <i>plumiger</i> Dön. <i>jessoensis</i> Tsuz. pupal type "A" Crawford, 1938. ✓ 2. <i>A. nigerrimus</i> Giles syn. ? <i>nero</i> Dol. <i>bentleyi</i> Bent. ? <i>pursati</i> Lav. ? <i>williamsoni</i> Baisas & Hu, 1936. var. X Venhuis, 1939. <i>venhuisi</i> Bonne-Wepster, 1951. pupal type "D" Crawford. 3. <i>A. indiensis</i> Theo. syn. pupal type "C" Crawford. 4. <i>A. minutus</i> Theo. (see p. 51). ✓ 5. <i>A. peditaeniatus</i> Leicester, 1908. syn. pupal type "E" Crawford. ✓ 6. <i>A. argyropus</i> Swell. 7. <i>A. lesteri</i> Baisas & Hu. syn. subsp. near <i>sinensis</i> of Colless, 1948. 8. <i>A. pseudosinensis</i> Baisas & Hu (see pp. 28, 48). ✓ 9. <i>A. crawfordi</i> , n. sp. syn. pupal type "B" Crawford. 10. Unnamed species near <i>nigerrimus</i> , provisionally called D2 (see p. 43).
TOTAL : 1 species, 2 varieties.	TOTAL : 10 species.

A. pullus Yamada, 1937, is probably another species, but it appears to be a Palaearctic form and not an Oriental one (see p. 52).

Previous work.

Most attempts to define varieties of oriental '*hyrcanus*' have been more or less unsatisfactory, because too few characters have been used, or only some of the stages in the life-history have been thoroughly examined. For example Swellengrebel (1914) described *argyropus* largely on the basis of the very broad hind tarsal bands but Barraud and Christophers (1931) found continuous variation in this character from narrow to very broad pale bands, and consequently they sank *argyropus* as a synonym of *nigerrimus*. We now see that although the broad white hind tarsal bands of

argyropus are its most striking character, the range of width of these bands overlaps with the range in *peditaeniatus*, and is not a reliable character for identifying the species, except in conjunction with the narrow mid-tarsal bands, and other characters.

In face of the bewildering amount of variation displayed by oriental '*hyrcanus*', some workers have been ready to believe that a number of species were present, whilst others have tended to dismiss it as a single highly variable species. Lamborn (1922a, p. 129) noticed that larvae and adults of '*hyrcanus*' in Malaya displayed much more variation than in China and Japan, and suggested that there might be a number of different forms, but he felt himself unsuited for investigations in systematics, and made no attempt to define such forms. On the other hand Hodgkin and Rajamoney (1933, p. 12), who limited themselves to distinguishing two varieties, *sinensis* and *nigerrimus* as defined by Edwards (1929) mainly on the presence or absence of a basal pale band on hind tarsus 4, wrote "... it is in fact difficult to avoid the conclusion that *sinensis* and *nigerrimus* are but one highly variable variety".

The general practice has been to follow Edwards (1929) and Christophers (1933) in recognising only two oriental forms, *sinensis* and *nigerrimus*; the former with a short dark mark at the base of vein 5, and narrow apical hind tarsal bands, the third not extending on to the base of segment 4; the latter with a longer dark mark on the base of 5, broader hind tarsal bands extending across the joints so that the base of segment 4 is pale, and often with a generally darker body colour than *sinensis*. But, as Christophers recognised, even this simple division does not hold good, and forms occur which have the *sinensis* type of hind tarsal banding but the *nigerrimus* wing, e.g., *lesteri*, some *peditaeniatus* in Assam, and a small proportion of *nigerrimus* Giles, over most of its range, and *vice versa*, e.g., *indiensis*.

Baisas and Hu (1936) were the first to define accurately any of the forms described here. They made a close study of the Philippine forms of '*hyrcanus*', and mainly on the characters of the eggs, larvae and pupae, distinguished three varieties: *nigerrimus*, *pseudosinensis* and *lesteri*. The last two had previously been identified as *sinensis*, but neither seemed to correspond to *sinensis* from China. Their *nigerrimus* was sufficiently distinct, and the description they gave allowed Venhuis (1939) to recognise it in Java, but the differences between *lesteri* and *pseudosinensis* larvae were very small, and the adults were not satisfactorily distinguished, so that the status of these forms appeared doubtful and they were not detected outside the Philippines.

The present study suggests that the following characters, or some of them, could be used to separate Philippine *lesteri* and *pseudosinensis*.

1. *Adults*: with pale scales on the mid coxae, and scales on the humeral cross vein; coxites of ♂ genitalia with pale scales; phallosome with two pairs of leaflets; ♀ palpi fairly uniformly shaggy, pale bands often not clearly defined; hind tarsal pale bands well marked. *Larvae*: inner clypeal hairs often with two or more branches distally, antennae short and stout with long sabre pieces; hairs 5 and 9 on abdominal segment VI usually with less than five branches; palmate hairs large with slender tips into which the pigment extends; pecten with about six long teeth. *Pupae*: rim of trumpet uniformly thin; hair 5 segment V with few (4-11) branches.....*pseudosinensis*
Adults: without scales on the coxae or the humeral cross vein; coxites of the ♂ genitalia without pale scales; phallosome with 5 pairs of leaflets; ♀ palpi tend to be narrow and less shaggy distally, with clearly marked pale bands; hind tarsal pale bands somewhat narrower. *Larvae*: inner clypeal hairs simple; antenna comparatively slender with short sabre pieces, hairs 5 and 9 of VI with 5 or more branches; palmate hairs smaller with blunter, unpigmented tips; pecten with about eight long teeth. *Pupae*: rim of trumpet with thickened and coarsely toothed areas.....*lesteri*

Crawford (1938) with remarkable insight distinguished five types of '*hyrcanus*' pupae in Malaya, but was unable to correlate these with differences in the adults, though he identified his type E with the *nigerrimus* of Baisas and Hu (= *peditaeniatus*), and type A with *sinensis* pupae from China as described by Baisas and Hu. Venhuis (1939) also considered that Crawford's type A was *sinensis* Wiedemann, and his type E, *nigerrimus* (*peditaeniatus*), but was not able to reach any definite conclusion about the remaining three types. Venhuis accurately described *nigerrimus* Giles naming it var. X.

The work of Baisas and Hu, Crawford, and Venhuis has been the starting point for the present study, which has been greatly aided by the fortunate circumstance that Malaya seems to be near the centre of distribution of the oriental '*hyrcanus*' complex so that most of the forms are present.

Interrelations.

The species described here fall into certain groups; *sinensis* and *nigerrimus* are closely related and differ from the remainder, particularly in quantitative characters (see Table III). *A. pseudosinensis* and D2 are related to *sinensis* and *nigerrimus*. *A. sinensis* is a mainland form, and *nigerrimus* may have been derived from it; the remainder probably originated on or near the Sunda shelf (see p. 50).

TABLE III.

Comparing the values of some quantitative characters of '*hyrcanus*'. Figures in brackets in the column heads are the numbers of specimens measured. See Tables X and XII for standard deviations, significance of differences, etc., for some of these characters.

Species	Adult				Larva		Pupa	
	Wing (50)		Mean number propleural setae (50)	Mean diam. Palmate IV or V, mm. (20)	Tergal Plate VIII (20)		Mean diam. spiracles mm. (5)	Paddle. Length of refractile border Total length (5)
	Mean length in mm.*	Width Length			Mean width mm.	Length Width		
<i>sinensis</i> ...	3.84	.234	6.9	.20	.30	.61	.084	.65
<i>nigerrimus</i> ...	3.82	.229	7.5	.19	.28	.62	.080	.67
<i>indiensis</i> ...	3.46	.224	3.7	.16	.24	.69	.067	.75
<i>peditaeniatus</i> ...	3.50	.223	4.1	.18	.25	.73	.073	.88
<i>argyropus</i> ...	3.56 ^a	.222	5.0	.18	.25	.73	.067	.87
<i>crawfordi</i> ...	3.79	.219	2.9	.14	.23	.71	.064	.65
<i>lesteri</i>	3.83	.216	3.9	.16	.26	.72	.073	.58

* The measurements were made on wild-caught adults; laboratory bred specimens are often rather small.

^a Only 32 specimens measured.

The Table shows that *sinensis* and *nigerrimus* are rather large, judged by wing length, and have the greatest number of propleural setae. The latter is evidently a real difference, and not merely a function of size as it tends to be in individual specimens, because the next largest species are *crawfordi* and *lesteri* which have about the smallest number of propleural setae. *A. crawfordi* and *lesteri* have the narrowest wings, and *sinensis* and *nigerrimus* the broadest. The latter pair have larger palmate hairs than the other species, and usually the tips of the leaflets are

more slender and the pigmentation extends further into them. The eighth tergal plate of *sinensis* and *nigerrimus* is large, and is wider than in the others, absolutely and relative to length; the spiracles also are larger. Three other characters that *sinensis* and *nigerrimus* have in common are the thin uniform rim of the pupal trumpet, contrasted with the non-uniform type with thickened, coarsely toothed areas on the rim (the general shape of the trumpet in the two groups probably differs a little, but this needs further study, especially in living pupae); comparatively numerous white scales on the mid coxae and eggs bluntly rounded at the ends with decks concave in side view.

A. peditaeniatus and *argyropus* clearly form a pair; the pupae of both are distinguished from the others by the reduction or absence of branches on the last (VIIIth) lateral spine, and the long refractile paddle border with well developed teeth. In the adult stage they resemble one another in a number of general features; they have the broadest hind tarsal bands, the scaling of the palps is similar, and the rather yellow to orange pale scales of the wing, and certain features of the pattern, are similar.

The general appearance of the adults of *A. crawfordi* and *lesteri* would not make one think that they are closely related, but the great similarity of the larvae and pupae shows that this is so, and in the quantitative characters given in Table III they are also very similar. The adult of *crawfordi* with its sharp bright wing pattern and eye spots on the mesonotum resembles *indiensis*, whilst the blurred and rather dark wing pattern of *lesteri* seems very different, but both have a narrow wing with a tendency to reduction of the apical fringe spot, no fringe spot at 5.2, and few or no scales on the humeral cross vein. Both have the *sinensis* type of hind tarsal banding and the leaflets of the phallosome are similar in number and shape.

A. indiensis is difficult to place; it seems to be somewhat intermediate between *nigerrimus* and perhaps *crawfordi*. The resemblance between the adults of the latter and *indiensis* has already been noted. In the width of the wing, the shape of the eighth tergal plate, and the rim of the trumpet, *indiensis* tends to be intermediate between the *sinensis*—*nigerrimus* group, and the remainder; whilst the presence of a pale band at the base of the third segment of the palps in the male, the pale scales on the basal half of the costa, the scales at the tip of the female abdomen, and the phallosome with only two or three pairs of leaflets, suggest affinities with *nigerrimus*.

Distribution and Evolution.

Distribution.

If the known distribution of the seven species described here is tabulated (Table XIV) or drawn on a map, it is evident that except for *sinensis* which belongs primarily to the continental mainland, these are species belonging at least as much to the Malay archipelago as to the mainland of Asia.

Although further collecting would probably show that the range of some of the species is greater than that indicated by the Table, the falling off in numbers on passing away from Assam, Burma, Siam, the Malay Peninsula and Sumatra, is fairly marked, so that these countries appear to form the endemic centre of the species. The diminution in species on passing eastwards through the archipelago can be explained by the frequent sea barriers to migration, but on the Asian continent climatic factors are the more probable barriers. The countries forming the endemic centre have a very uniform temperature which, apart from altitude, does not vary more than about 10 degrees above or below 80°F. throughout the year, whilst annual rainfall is 80 ins. or more over most of the area, especially along the coast and in the hills (Dobby, 1950). High maximum temperature and dry periods might well be the barrier preventing *sinensis* from spreading into India from Assam, and conversely low

minimum temperature may be the factor limiting the spread of *indiensis* and *peditaeniatus* from Indo-China into China.

TABLE XIV.
Distribution of the species.

Countries	<i>sinensis</i>	<i>nigerrimus</i>	<i>indiensis</i>	<i>peditaeniatus</i>	<i>argyropus</i>	<i>lesteri</i>	<i>crawfordi</i>	Total
China ...	+			?+*				2
Indo-China ...	+		+	+				3
India and Ceylon ...		+	+	+	?+			4
Assam, Burma, Siam ...	+	+	+	+	+			5
Malay Peninsula ...	+	+	+	+	+	+	+	7 (plus D2)
Sumatra ...	+	+	+	+	+		+	6
Java ...		+		+	+			3
Borneo ...		+	+	+		+		4
Philippines ...				+		+		2 (plus <i>pseudo-sinensis</i>)
Celebes and Moluccas ...		+		+				2

* Ho (1938) records *nigerrimus* from Hainan Island; on grounds of the characters given by him and the distribution recorded here, this seems more likely to be *peditaeniatus* than *nigerrimus*. Sweet & others (1941) record *nigerrimus* from S.W. Yunnan, but the locality is almost in Burma, and both *nigerrimus* and *peditaeniatus* may well be present.

The endemic centre of these '*hyrcanus*' species is rather similar to that of the *Anopheles umbrosus* group (Malay Peninsula, Sumatra and Borneo, Reid & Hodgkin, 1950) but shifted somewhat to the north towards the continental mass, so that Assam, Burma, and Siam are more important than Borneo. These '*hyrcanus*' are therefore not so strictly Malaysian as the *umbrosus* group, which is much more dependent upon evergreen rain forest conditions.

Relation to palaearctic forms.

Towards the periphery of their ranges on the continent, these southeast Asian '*hyrcanus*' species are replaced by other members of the *A. hyrcanus* complex. About 6-8 palaearctic forms are recognised which between them have an enormous range stretching from North Africa and Spain in the west, through Italy and Greece to the Black Sea and the Caspian, and down into Mesopotamia and Arabia, and eastwards from the Caspian and Aral Seas through Kazakhstan and Mongolia to the valley of the Amur river on the north Pacific and then southwards into China and Japan (Bates & others, 1949). Christophers (1933) records *nigerrimus* from the northwest frontier of India, but it is quite likely that neither *peditaeniatus* nor *nigerrimus* extend so far west. Theobald's type of *minutus* from the Punjab may be an example of a northwest Indian form that replaces *nigerrimus*; it is certainly different from that species though having some points of resemblance to it. The banding of the palps of *minutus* tend towards the form seen in var. *mesopotamiae*, and other palaearctic forms of the *hyrcanus* group, in which the basal bands are better marked than in oriental forms, and the band at 3/4 is as broad as that at 4/5 (Christophers, 1924, 1933). Christophers and Chand (1915), and Edwards (1921) noted resemblances, chiefly in the wings, but also in the banding of the palps between *mesopotamiae*

from Arabia and Mesopotamia on the one hand and *sinensis* from China, eastern Siberia and Japan on the other. This suggests that the name *sinensis* perhaps covers more than one species, for the *sinensis* from Malaya described here, and shown to be probably the same as Wiedemann's *sinensis* from South China, is certainly of the oriental form, with the last two pale bands of the palps broader than the more basal ones. This holds good for most of the *sinensis* from China that were seen, but out of 38 ♀ specimens examined from Hong Kong in the South to Mukden (Manchuria) in the north, five or six were found which had the palaearctic type of banding, and which seemed to differ in other ways. Five out of these six were from Nanking (32°N.), or further north (see list of specimens p. 23), the sixth which may have been of the same form, was from further south from a place about 200 miles W.N.W. of Chungking but at an altitude of 2,000 ft., where height and inland position might be expected to produce continental extremes of climate. No clear cut differences between the oriental and palaearctic forms could be discovered, but the examination was limited by the small number of specimens (adults only) and insufficient time. However, the following tendencies were noted. The northern form is very large with the blurred smoky wing pattern remarked upon by Edwards and resembling that of *mesopotamiae*. The pale scales may be very numerous both on vein 1 and the posterior border of the costa, but they are dull and tinged fuscous, except for the subcostal spot which is bright and long. The dark scales are not sharply black and the apical fringe spot is dull or obsolete. The palps distal to the second segment are thin, not very shaggy (see Christophers and Chand, Pl. 15, fig. 2), and the pale bands are rather narrow, but clear and of more or less equal width. Other possible characters were noted such as a tendency to have pale scales flanking the dark scale tuft on the seventh abdominal segment beneath, or some pale scales on VIII.

The oriental *sinensis* in China is very variable in size and appearance; it occurs together with the palaearctic form at Nanking, and further north apparently at least as far as Tsinan, 38°N. Compared with the palaearctic form, it usually has a clear bright wing pattern, with dark and pale scales well contrasted, a bright apical fringe spot, and shaggy palps which taper uniformly towards the tip where the apical two pale bands are usually broader than the basal two, and may fuse, and pale scales are often scattered between the bands. The fore tarsal bands are perhaps broader, and the plume scales on the wings may be a little less slender.

There is other evidence that there are at least two forms of *sinensis* in China. Walch and Walch-Sorgdrager (1935) found two types of eggs amongst material from Nanking; most were the very wide decked type which appears to be that of the true *sinensis* Wied. (p. 20), but there were a few with much narrower decks. Yao and Wu (1935) working on *sinensis*, also at Nanking, found only the narrow decked type which is presumably the egg of the northern form. Differences in seasonal distribution,* breeding places, and chance factors, could account for one pair of authors having predominantly wide decked eggs, and the others obtaining only the narrow decked. Feng (1938) describes a great variety of breeding places and remarks on the variability of the species, and Yao and Wu (1936) note variability in the male genitalia. *A. pullus* Yamada (1937) is perhaps the Korean peninsula representative of the northern form, from which it is said to differ in a number of small points such as usually seem to distinguish these 'hyrcanus' species. Yamada describes the egg as having a deck one-fifth as wide as the egg, which makes it distinct from both the southern form (*sinensis* Wied.) with a deck one-third to one-half as wide, or the northern form which is illustrated by Yao and Wu with a deck one-seventh to one-

* I have recently seen a series of *sinensis*, collected month by month at Nanking in 1933, in the Department of Parasitology (Dr. A. A. Sandosham), University of Malaya, Singapore. Both oriental and palaearctic forms seemed to be present, and examination suggested seasonal variations in relative abundance. Amongst specimens collected in July, 13/17 appeared to be the oriental type, compared with 6/18 in October.

tenth as wide as the egg. The wing of *pullus* lacks any fringe spot at apex or 5.2, there are no scales on the humeral cross vein, the remigium is entirely pale scaled, the ventral scale tuft on segment VII in the female is all black, and the male coxites are white scaled laterally as well as dorsally. The mainland northern form is described by Yamada as having a small dull fringe spot at 5.2, and the apical fringe spot entirely lost (this agrees with my observations), the humeral cross vein bears pale scales, and the remigium is partly dark dorsally. The ventral scale tuft on segment VII in the female is often surrounded by white scales, and the male coxites are dark scaled laterally. The larval breeding places of the two forms are said to differ.

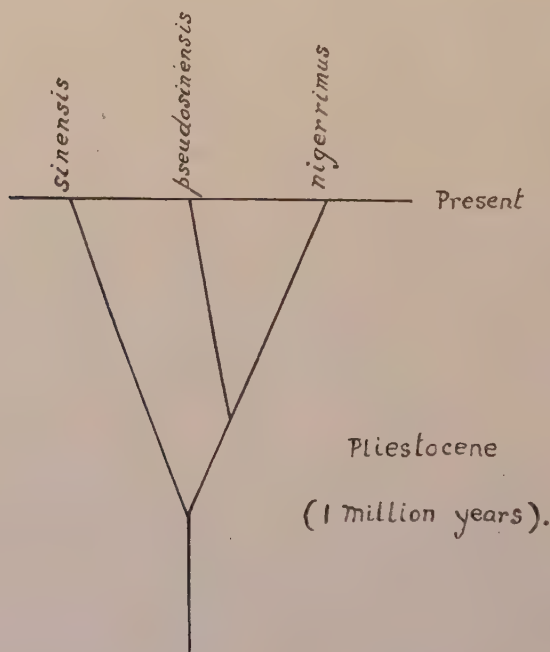
The relation of the island forms of '*hyrcanus*' in Japan and Formosa to those on the mainland is a separate problem. Some differences due to isolation are to be expected, but the mainland forms require thorough study, before any such differences could usefully be described. It is quite likely that the continental forms will be found to be less sharply distinct from one another than the southeast Asian forms, and seasonal differences in size and appearance might be a further complicating factor. Seasonal differences in the eggs of *sinensis* in Japan are reported by Otsuru and Miyagawa (1950). The southeast Asian forms are fully sympatric, whilst the continental forms may prove to be subspecies (allopatric) which replace each other in different parts of Eurasia with zones of overlap and perhaps of hybridisation where they meet. Only a thorough study of large amounts of material in all stages will reveal the real situation. If the pattern is similar to that of *A. 'maculipennis'* (Bates & others, 1949), we may expect a number of species overlapping widely, and some of them polytypic; *i.e.*, with two or more subspecies occupying different geographical areas.

To sum up, it appears that the '*hyrcanus*' complex consists of at least two major groups which probably have some small morphological differences distinguishing them, though it remains to be seen whether they are sharply distinct. The southeast Asian group described here consists of sympatric species adapted to the rather uniformly warm moist conditions of the region, and giving place near the boundaries of that region to palaearctic forms. The palaearctic forms constitute the second group adapted to quite different climatic conditions; they require much further study. The *A. coustani* complex in Africa is possibly a third group.

Speciation.

A large part of the range of the southeast Asian *hyrcanus* group is formed by the broken land area of the Malay archipelago, which has been subject to many changes in fairly recent geological history, making it a very favourable area for the development of species by geographical isolation. Thus it is not surprising to find that the species described here seem to have arisen in this manner. The relation between *sinensis*, *nigerrimus* and *pseudosinensis* is a good example of this. As explained on p. 28, structural features show that *pseudosinensis* is intermediate between *sinensis* and *nigerrimus*, but is more directly related to the latter. The present distribution of these forms also points to this; *nigerrimus* is found in Borneo and Celebes, but *sinensis* appears to be absent. Since there is no reason to think that the Philippine '*hyrcanus*' fauna could have been derived direct from the mainland of China, and the Philippine fauna as a whole is usually considered to be closely related to the fauna of the Malay archipelago, derived in large part via Borneo and Celebes, it seems impossible for *pseudosinensis* to have been derived direct from *sinensis* as we know it to-day, but perfectly possible for it to have been derived from *nigerrimus*. But since *pseudosinensis* is intermediate in many characters between *sinensis* and *nigerrimus*, it is probably descended from a proto-*nigerrimus* which reached the Philippines and was isolated there at a time when *nigerrimus* as we see it now did not exist. This seems more likely than the alternative possibility

that the present form of *nigerrimus* was isolated in the Philippines and reverted in character towards *sinensis*. Reasoning becomes more speculative if one tries to trace the process further back, but the present distribution of the three species and the fact that large fluctuations of surface area occurred in Sundaland during pleistocene times suggest that originally a *sinensis*-like form entered the archipelago from the north via what is now Siam and Malaya. The land bridge was then perhaps broken, or much narrowed by a rise of sea level due to melting of the ice caps during an inter-glacial period (Zeuner, 1941, fig. 1). This *sinensis*-like form continued to spread and evolve in the archipelago, and reached the Philippines via Palawan* (there giving rise to *pseudosinensis*) during a glacial period when the fall in sea level had exposed all the land of the Sunda shelf, and the Philippines were but little separated (Zeuner, fig. 2). Towards the end of this period, or on a later occasion, there was another invasion from the north, and the modern *sinensis* entered the Malay Peninsula and Sumatra, but by that time the *sinensis*-like form had evolved to *nigerrimus* which was too distinct for crossing to occur, so that their ranges now overlap widely. The suggested relation is summarised in the diagram.



The differentiating effect of geographical isolation is also shown by *lesteri*; the type form in the Philippines shows one or two small but definite differences from the form inhabiting Borneo and the Malay Peninsula (see p. 41), and the two might be regarded as subspecies.

In passing it is interesting to note how the close study of a group of animals, in this case a sibling species complex among Anopheline mosquitos, confirms the geological evidence that the Philippines are much more isolated from Borneo than the latter is from the Malay Peninsula. Linear distances on a political map show Borneo

* According to Baisas and Hu neither *pseudosinensis* nor *lesteri* occur in Mindanao which seems to exclude the Jolo islands as a route of entry.

connected to the Philippines by two strings of islands, but separated from the Malay Peninsula by a wide sea gap with few islands. But if one looks at a map such as that given by Mayr (1945) or Zeuner (1941), showing the edges of the continental (Sunda) shelf beneath the sea (200 metre line), one sees that Borneo is on the Sunda shelf separated from the Malay Peninsula, Sumatra and Java only by shallow seas seldom more than 100 metres in depth (Scrivenor & others, 1944) which have been dry land at least once during pleistocene times. Deep water channels, which were never entirely dry land during those times, separate the Philippines from Borneo. If there were sufficiently reliable evidence about the dating of the main fluctuations in sea level and land area in the archipelago during pleistocene times, a study of such pairs as *nigerrimus* and *pseudosinensis* and the two forms of *lesteri*, which have one form common to the Malay Peninsula and Borneo, and the other found only on the Philippines, might allow us to form some idea of the rates of speciation.

In addition to the two examples of morphological differentiation by geographical isolation discussed above, geographical variation has been noted in *peditaeniatus*, *nigerrimus*, *argyropus* and *sinensis*. In the latter at least, it appears to be more or less continuous (clinal), not abrupt, and the distribution of this species is mainly continental and continuous. In short, the study of the *hyrcanus* group in southeast Asia suggests that speciation has mostly been by the usual process of geographical isolation and subsequent differentiation (Mayr, 1947), the species conforming to the classical pattern of sympatric species (allopatric in origin) each of which may differentiate into infra-specific forms occupying different areas. However, there may perhaps be exceptions to this process. It seems possible that where a species of *Anopheles* has split into salt water and fresh water forms, these may have differentiated in consequence of ecological rather than geographical isolation. Thomson (1951) has shown that salt water forms of *Anopheles gambiae* have arisen independently on the west and east coasts of equatorial Africa. On the west coast the salt water form is considered to be a distinct species, *A. melas*, distinguished from *gambiae* by small differences in the eggs and larvae, as well as by biological differences. On the east coast no morphological differences were found, but there were differences in biology, and the females could be identified by the reactions of their larval progeny to salt water. Larvae of the salt water form, if hatched in fresh water and then placed in 75 per cent. sea water, lived at least six hours; larvae of the fresh water form were all dead within two hours. This picture of salt water forms of *A. gambiae* arising independently on two occasions with ranges separated by the whole width of Africa, and differing from the parent fresh water form mainly in their adaptation to salt water, morphological differences being very small or absent, certainly suggests, though it does not prove, that salt water (an ecological factor) has been the principal differentiating agent. The possibility remains, however, that the adaptation to salt water took place in comparative isolation on islands off the coast where scarcity of suitable fresh water encouraged *A. gambiae* to invade brackish water. If the salt water forms of *gambiae* have in fact arisen as the result of ecological and not geographical isolation, it is possible that other brackish water species such as *A. baezai* and *sundaicus*, or even *lesteri*, might also have done so, even though they may now show evidence of geographical variations.

In considering the origin of habits, there seems to be some support for Pittendrigh's contention (1950) based on his study of the bromeliad-breeding Anophelines, that behaviour differences may be developed, or at least greatly accentuated, sympatrically, after the species (allopatric in origin) have come into competition. For example, in the Philippines where there are only three species of the *hyrcanus* group, *lesteri* and *peditaeniatus* are found both on the coast and inland (Baisas & Hu, 1936). However, in the Malay Peninsula (Selangor) where there are seven species, *lesteri* is very rarely found except on the coast near or in brackish water, whilst *peditaeniatus* is an inland species not yet found near brackish water.

If one assumes that degree of dispersion may be an indication of age, *peditaeniatus* and *nigerrimus* are possibly the oldest species. They have crossed the Wallace line, and *nigerrimus* has crossed the Weber line also, being found on Boeroe in the Moluccas. It would be unwise to pursue this line of reasoning further, until much more collecting has been done. The ranges of *lesteri* and *crawfordi* are probably considerably greater than those recorded here, so that it would be wrong to assume that they are the youngest species merely because their present known ranges are the smallest. Also the habits of the species may greatly affect their rates of dispersion.

Biology and Relation to Disease.

There has not been time to make a thorough study of the literature, and it is uncertain to which of the species many of the records refer. Little, therefore, is said about the biology of '*hyrcanus*', outside Malaya, and what follows should be regarded as no more than notes on the fragments of information about the habits of the different species, which is all that we have so far.

Broadly speaking the habits of '*hyrcanus*' are everywhere much the same. The larvae are found in swamps, rice-fields and similar situations in the open, and the adults bite cattle for preference, but will also bite man. Within this broad framework there are considerable variations in habits, and these may be sufficient to make one species quite often a vector of malaria or filariasis, whilst another is harmless; in this respect the group resembles *A. 'maculipennis'*.

Larval breeding-places.

Although all '*hyrcanus*' are found typically in swampy breeding places, *i.e.*, still or slowly moving water with vegetation, there are subdivisions within this general type. In Malaya '*hyrcanus*' larvae are found in most kinds of swampy situations, except jungle swamp, but it is evident that the different species have different preferences, though it is not very clear yet what these are, and there is overlapping since several species may be found in the same breeding place. *A. sinensis* is found commonly in open grassy ponds and such places, sometimes on the coast with *A. sundaicus*. *A. nigerrimus* seems to prefer deep ponds or swamps in which the water surface is largely covered by plants. For example, there are some ponds at Kepong near Kuala Lumpur in which Chinese keep fish and grow *Pistia stratiotes* for pig feed. The *Pistia* forms a complete cover on the water surface, and *nigerrimus* has been the dominant form of '*hyrcanus*' in these ponds for many years. Venhuis (1939) says of *nigerrimus* (his var. X) "The breeding places are mostly rather deep swamps and swampy ricefields with much vegetation like *Azolla pinnata*, *Pistia stratiotes*, *Jussieuia repens*, *Hydrilla verticillata*, *Eichhornia crassipes*, *Spirodela polyrrhiza*, etc." The form related to *nigerrimus* and provisionally labelled D2 was found breeding in company with *nigerrimus* and *A. ramsayi* in *Pistia*-covered ponds in north Kedah (Reid, 1950). *A. indiensis* and *peditaeniatus* seem to be the commonest '*hyrcanus*' in ricefields though found in many other places as well. *A. argyropus* is the least common of the species in Malaya, but may be locally very abundant as in the Pekan-Kuantan area of Pahang where there are extensive swamps. Near Kuala Lumpur the larvae have been found in a group of large swamps and pools, at points where bushes and reeds grow in the water and provide considerable shade. *A. lesteri* in Malaya is almost confined to the coast, though one or two adult specimens have been caught in Kuala Lumpur showing that it can breed inland. In the Philippines it is found inland as well as near the sea. On the Malayan coast it is often found in shady places, sometimes in company with *A. baezai*, but probably occurs also with *sundaicus* in less shady situations. Hodgkin (1940) found that '*hyrcanus*' bred freely in water with a salinity of about 18 per cent. of that of sea water, and obtained it once from water 75 per cent. as salt as the sea. His records probably refer to

sinensis as well as *lesteri*. Capt. C. E. Shearman, R.A.M.C., who kindly donated specimens, has collected *lesteri* from breeding places in Singapore with salinities as high as 82 per cent. sea water. The short anal papillae of *lesteri* presumably reflect the salinity of the breeding place (Wigglesworth, 1942, p. 284). If specimens could be obtained from an inland locality, the anal papillae would probably be no shorter than in the other forms. The anal papillae of *A. sundaicus* and *baezai*, both salt water species, are also short. *A. crawfordi* is widely distributed but generally less abundant than the other species; the larvae have been found in deep or shallow swamps shaded by tall emergent grass and other vegetation beneath which they shelter.

Thomson (1950) showed that the thermal death point of '*hyrcanus*' larvae in Assam was 43.0–43.5°C. with five minutes' exposure, and the larvae survived exposure for an hour at a temperature of 40°C. *A. minimus* on the other hand was always killed by five minutes' exposure to 41°C. These differing thermal death points were evidently adaptive, and related to the temperature of the natural breeding places. *A. minimus* bred at the shaded edges of running water where the temperature seldom exceeded 35°C., but *A. 'hyrcanus'* was found in ricefields where the temperature often exceeded 41°C. It seems quite likely that a careful study would show differences in the thermal death points and temperature ranges of the breeding places, for the different members of the *hyrcanus* group. *A. sinensis*, *indiensis* and *peditaeniatus* might be expected to be more tolerant of high temperatures than *nigerrimus*, *crawfordi* or *argyropus* which seem to prefer some shade in the breeding places.

Larval colour pattern.

Lamborn (1922a) remarked on the great variety of colour and pattern to be seen in '*hyrcanus*' larvae in Malaya, compared to those of China and Japan which were nearly always green. The range of appearances is certainly striking, and in part reflects the number of different species present, but the variations encountered within one species are so large that larval identification by the colour pattern will probably seldom be possible. Black "*hyrcanus*" larvae with bright white bars on the abdomen are probably those of *nigerrimus*, but this species may also be green. The limited observations made so far suggest that transverse pale bands on the abdomen (segment III, V and VIII) are characteristic of *nigerrimus* and may sometimes be found in *sinensis* (they are not uncommon on *sinensis* larvae in China; Lamborn, 1922a), but are absent in the other species, in which pale abdominal markings, if present, tend to be in the form of a median longitudinal line (*lesteri* and *crawfordi*). The general body colour varies from green through yellow and brown to black; *sinensis* seems usually to be green, whilst possibly *nigerrimus* is the only species which is often really black, but for the most part each species can span the colour range from green to black.

Probably environment is an important factor in determining the colour; the impression has been that larvae from open sunny breeding places are pale coloured (commonly green), but that they are brown or dark brown from shady places. *A. argyropus* and *crawfordi* seem to choose shady places and they are generally dark brown. In an attempt to see whether colour is closely correlated with species, larvae were grouped according to colour and the resulting adults identified. It happens that only *sinensis* and *nigerrimus* larvae were obtained in useful numbers; the results were as follows:—

TABLE XV.
Larval coloration.

Species	Green	Brown	Dark brown,	Black
<i>sinensis</i> ...	72	9	0	0
<i>nigerrimus</i> ..	51	10	2	22

These figures seem to support the belief that *sinensis* is usually green, and suggest that *nigerrimus* tends to be green or black, with intermediate shades less common. However, more collecting from a wider range of breeding places would be needed to establish this.

The impression from these incomplete and sketchy investigations is that larval colour pattern is probably partly genotypic in origin, *e.g.*, the white bands or lines, and partly phenotypic, *e.g.*, general coloration. The phenotypic factors might be food, or the colour and amount of illumination of the environment. Lamborn (1921) suspected that environment was the more important.

Adult biting behaviour.

Covell (1944) says that the adults will bite man out of doors in the evening, and in the shade during the day. Malayan experience agrees with this summary. If men are employed to catch out of doors on their bare legs, '*hyrcanus*' will attack freely shortly after dark, but is usually reluctant to bite men indoors. The '*hyrcanus*' species will enter a man-baited net trap beneath a house or on an open verandah, but *lesteri* and *indiensis* at least, will not enter a window-trap hut with narrow louver entrances, unless the hut contains animals (a calf or goats) instead of man. By contrast, the important Malayan vectors of human malaria such as *A. maculatus* and *barbirostris* (dark winged) will enter man-baited window-trap huts, though they may also be attracted to animals. *A. 'hyrcanus'* is considerably more zoophilic than these species, and is usually common at cattle sheds; it tends to bite immediately after dark and is less numerous after about 9 p.m. (Inst. med. Res. Malaya, 1951, ? *indiensis*). Occasional specimens will attack in shady places during the day. A few *lesteri* have been caught in this way when disturbed in their daytime resting places.

Daytime resting places.

In common with most mosquitos in Malaya, '*hyrcanus*' is seldom found in houses by day. Wharton (1950) found '*hyrcanus*' (mostly *indiensis*) resting low down in company with *maculatus*, *philippinensis* and other species, amongst ferns growing beneath light shade cast by mature rubber trees. *A. lesteri* has recently been found regularly in company with *A. hackeri* in hollows low down between the bases of old Nipah palm fronds at a place on the Selangor coast. At the same place *A. baezai* is found resting on the growing Nipah palm fronds up to about four feet above the ground where it is easily seen. The resting attitude of *lesteri* seems to be typical of most of the series *Myzorrhynchus*; the body is at right angles, or nearly so to the vertical surface on which the mosquito is resting, and the hind legs are held out at right angles to the abdomen, and in the same horizontal plane, with the tarsi curving forward. *A. hackeri* and *kochi* hold the hind legs together directed downwards beneath the abdomen, with the tarsi curving upwards.

Swarming and mating.

Wharton (Inst. med. Res. Malaya, 1950) has observed *indiensis* swarming and mating at dusk. The swarms have usually been six to eight feet above the ground, over the top of some small bush.

Attraction to light.

A. indiensis and *peditaeniatus* have been caught resting at night on a split bamboo verandah wall in the circle of light cast by a kerosene pressure lamp.

Relation to malaria.

Anopheles 'hyrcanus' (sinensis) has long been regarded as the principal or only vector of malaria in some parts of China. Chow (1950), in a brief review, says that it is the principal vector in central China, but in south China is replaced in this role by

minimus and in north China by *pattoni*. On Formosa it is the vector in the plains, being replaced by *minimus* in the foothills. In southeast Asia proper, '*hyrcanus*' is overshadowed by more important vectors such as *minimus*, *culicifacies*, *maculatus*, *sundaicus*, *leucosphyrus* and others. Nevertheless, the records show that it has been responsible for a number of local epidemics of malaria. Covell (1944) records infections from Java, Sumatra, Malaya and Indo-China, and McArthur (1950) lists a few gut infections in '*hyrcanus*' from Borneo. The question arises as to which species of the group these records apply, for it is not likely that all are equally liable to transmit malaria. In most cases it is impossible to tell now which species were found infected; the records refer to both "*sinensis*" and "*nigerrimus*" but this does not necessarily mean that more than one species was involved, for one species might have been, and sometimes was identified as *sinensis* or *nigerrimus* or both according to the criteria employed. There is evidence that *nigerrimus* has been a vector in a number of outbreaks of malaria, but except for *sinensis* in China, there seems little evidence at present to incriminate any of the other species. Venhuis (1939) found that *nigerrimus* (his var. X) was responsible with *barbirostris* for an outbreak of malaria at Benteng in Celebes, and during an epidemic in the Karangbinangoen district of Java he found it infected and considered it to be the principal vector. The ratio of gland to gut infections was low, however, and he says "Probably the density of *A. hyrcanus* X (= *nigerrimus*) must be heavy before it can give rise to an outbreak of malaria". Van Hell (1950) holds the same opinion, and records further infections in this species from south Celebes.

Hodgkin (1933) found that '*hyrcanus*' was responsible for an outbreak of malaria in the Brickfields road area of Kuala Lumpur, near to which there were some large swamps. He dissected over three hundred '*hyrcanus*', of which about two-thirds were identified as var. *nigerrimus* and one-third as var. *sinensis*, and found gut and gland infections in both varieties. Only negligible numbers of other species of *Anopheles* were caught. In 1941 eighteen specimens of '*hyrcanus*' were found in the collection of this Institute, which had been caught in a human-bait net trap during these investigations at Brickfields Road. These specimens had been captured on three different nights (17, 18 and 26.vi.1931), and all were *nigerrimus*. Probably, therefore, this species predominated and was the vector, and most of the specimens identified as *sinensis*, were *nigerrimus* with narrow hind tarsal bands.

There are good reasons, therefore, for regarding *nigerrimus* as a potentially important vector of malaria in southeast Asia, at least when it is abundant close to a dense human population. In India, however, it seems to be unimportant, for no infections have been found in "*hyrcanus*" (Covell, 1944). *A. sinensis* in China, and perhaps also Indo-China, is the only other species of the group that has been proved to be a vector, but since there appear to be two forms included under this name in China, the exact identity of the vector remains in some doubt.

Relation to filariasis.

Human filariasis and elephantiasis in southeast Asia are of two kinds. One, due to *Wuchereria bancrofti*, has a world-wide distribution in warm countries, and can be transmitted by many different mosquitos, including *Anopheles*. The second kind, due to *W. malayi*, is largely restricted to southeast Asia, and typically is transmitted by mosquitos of the genus *Mansonia* (subgenus *Mansonioides*), less often by the larger species of *Anopheles* such as *barbirostris* (Wilson & Reid, 1951).

In China *A. 'hyrcanus'* (*sinensis*) has been found naturally infected with *W. bancrofti*, and experimentally has proved to be easily infected with *W. malayi* (Chow, 1950). In Malaya "*bancroftian*" filariasis is uncommon and there can be little transmission; in any case the most likely vector, which is also the main one in surrounding countries, is *Culex fatigans*, not *A. 'hyrcanus'*. With "*malayan*" filariasis the situation is different; there are a number of areas of high endemicity in

Malaya where transmission is intense, and although the principal vectors are species of *Mansonia*, recent work described below has shown that *A. barbirostris* and 'hyrcanus' play a part.

Poynton and Hodgkin (1938) in Malaya found natural infections only in species of *Mansonia*. They were concerned mainly with the larger endemic areas of filariasis on the lower reaches of the Pahang, Perak and Bernam rivers, where *M. longipalpis*, breeding in great tracts of swampy jungle, was the main vector. Hodgkin (1937, 1939) obtained experimental infection rates of 80 per cent. and upwards in species of *Mansonia*, but only low rates with *A. barbirostris* (3 per cent.), *hyrcanus* var. *sinensis* (1 per cent.) and var. *nigerrimus* (17 per cent.).

In the last few years investigations have been made in open settled rice-growing areas near the coast in Kedah and Province Wellesley. In these areas *Mansonia longipalpis* is uncommon, and the principal vectors appear to be *M. indiana*, *uniformis* and *annulifera*. *A. 'hyrcanus'* has formed a considerable proportion of the mosquitos caught, and out of about 1,000 'hyrcanus' dissected, five have been found infected with larvae presumed to be those of *W. malayi*. The specimens dissected belonged to four or five species of the *hyrcanus* group, chiefly *sinensis*, *nigerrimus*, *lesteri* and *peditaeniatus*, but four out of the five infected specimens were *nigerrimus*, one of which contained full-grown infective larvae. The fifth specimen was identified as *lesteri*, but as the rather similar form described here as D2 had not been recognised at that time, but was subsequently found to occur in the area, the identification is uncertain.

The results of attempts to obtain experimental infections have been very interesting. Mosquitos were fed about 9 p.m. each night on an infected volunteer who had about 300-400 microfilariae of *W. malayi* per 60 cu. mm. of blood. The results with *Mansonia* spp. agreed well with those of Hodgkin; infection rates of 80 per cent. and upwards were obtained, and full grown infective larvae were first found 8½ days after the infective meal. With *A. barbirostris* (light and dark winged forms), and the *hyrcanus* group, the infection rates were very different from Hodgkin's. Both forms of *barbirostris* were readily infected with rates of over 80 per cent., though the average number of larvae per mosquito (10) was lower than in *Mansonia* (20). In the *hyrcanus* group, the infection rate varied widely according to the species; the figures are shown in Table XVI.

TABLE XVI.

Results of applying various species of the *A. hyrcanus* group to a human volunteer infected with *W. malayi* (300-400 microfilariae per 60 cu. mm. of blood).

Species	No. applied	No. fed	% fed	No. dissected	No. infected	% infected
<i>sinensis</i>	184	118	64	99	1	1
<i>nigerrimus</i>	200	172	86	154	69	45
<i>indiensis</i>	93	48	52	35	11	31
<i>peditaeniatus</i>	87	51	59	43	38	88
<i>lesteri</i>	126	99	78	96	38	40
<i>crawfordi</i>	8	5		5	5	—

The main points of interest in the Table can be discussed in order. *A. sinensis* is evidently very refractory to infection, although it bites fairly readily. A few specimens were found with large numbers of dead chitinated first-stage worms in the thoracic muscles, which clearly seems to indicate that *sinensis* in Malaya is an unsuitable host. Presumably the var. *sinensis* with which Hodgkin worked was this form for he also obtained only a one per cent. infection rate. This resistance to

infection seems anomalous in view of the ease with which *sinensis* has been infected experimentally with *W. malayi* in China, but one cannot be sure that the *sinensis* used there was the same as the Malayan. We have seen that there are probably two forms in China confused under the name *sinensis*. Even if it were known that the experiments had been made with the type form with which the Malayan appears to be conspecific, there are still minor morphological differences between Malayan and Chinese *sinensis*, and there seems no reason why there should not also be physiological differences.

A. nigerrimus was not very readily infected, and full grown larvae were not found earlier than 10½ days after the infective meal; the average number of worms per infected mosquito was only two. However, *nigerrimus* had the highest biting rate, and this is probably a significant point since the species has been found to be a natural vector of malaria and filariasis. Experience suggests that readiness to bite man is frequently a much more important factor than infectability in deciding the vector status of a species, at least where malaria is concerned. For example *A. kochi* is readily infected with the parasites of human malaria, but does not transmit the disease in nature, apparently because it is too little attracted to man. On the other hand *A. letifer* and *barbirostris* (dark winged) are efficient natural vectors, but very difficult to infect experimentally (Reid & Hodgkin, 1950).

Kariadi (1941) implicated *nigerrimus* (=var. X Venhuis) in the transmission of malayan filariasis at Martapoera in southeast Borneo. He found it less readily infectable than *Mansonia uniformis*, and development of the full grown larvae was slower, but on account of its large numbers and willingness to bite man he considered it important.

The remaining species in Table VI do not call for much comment. *A. indiensis* seems unlikely to be a vector in nature for it has both a low biting rate and a low infection rate. *A. peditaeniatus* is as readily infected as *barbirostris*, but the biting rate is rather low. *A. lesteri* has a higher biting rate, and although it is reluctant to enter a man-baited hut (p. 58) it should be regarded with suspicion. The numbers of *crawfordi* are too small to draw any conclusions from them, and *argyropus* has yet to be tested.*

Discussion.

Apart from the straightforward systematic revision and any practical value which that may have, it is hoped that this study will serve to indicate the size, complexity and interest of the *A. hyrcanus* species group, and will suggest to others that further study might throw considerable light on problems of speciation and spread in Anopheles mosquitos. Some of the immediate problems that call for further investigation are the probability that there are two forms of *sinensis* in China, an oriental (presumably Wiedemann's type form) and a palaearctic, which overlap to an unknown extent and may perhaps interbreed. Arising from this, there is the broader question of how the palaearctic forms are related to the oriental ones not only in morphology, but also in space and time, and this question would require the study of *hyrcanus* in northwest India as well as in China. The exact relationship between *hyrcanus* and *coustani* would also bear further investigation. Within the oriental region, detailed study of geographical variations in morphology and biology, in addition to furthering the studies of speciation, would probably explain why some species such as *nigerrimus* and *sinensis* are vectors of disease in one area and not in another.

* Three *A. argyropus* were applied to another carrier at a later date. Two fed and became infected.

Summary.

This paper is a study of the southeast Asian forms of the *Anopheles hyrcanus* complex with special reference to those of the Malay Peninsula, and it is to the latter forms that the detailed descriptions apply. The group is distinguished from all but a few other species by the presence in the adult female of a tuft of scales on the clypeus on each side, together with pale bands on the palps and a ventral tuft of scales on the seventh abdominal segment. For a complete diagnosis see page 6.

The usual practice has been to recognise only two oriental forms of *hyrcanus*: varieties *sinensis* and *nigerrimus*. It is shown that these varieties as recognised up till now are heterogeneous, and that at least in southeast Asia, '*hyrcanus*', is a group of sibling species of which there are no less than seven or eight in the Malay Peninsula. For convenience, and where the exact species is not known, the name '*hyrcanus*' in inverted commas is used, meaning *hyrcanus* sibling species group.

There is no doubt that these sympatric forms are distinct species for although individual characters, such as the width of the pale bands on the hind tarsi, are variable and the range of variation between two or more species may overlap, by using a combination of characters there is no difficulty in identifying individual adult specimens of either sex. Furthermore, offspring reared from eggs are always of the same form as the female parent. Larvae, pupae, and eggs can usually be identified, and keys are given for the identification of these stages as well as the adults.

Seven named species are described, but only one of these, *A. crawfordi*, is regarded as new, the others have all been described at various times, and the names later sunk as synonyms of *sinensis* and *nigerrimus*.

All seven species have been recognised also outside the Malay Peninsula, and some have a wide range from western India to the Philippines or Moluccas. Except for *sinensis*, the species belong to southeast Asia, where Assam, Burma, Siam, the Malay Peninsula and Sumatra support the largest number. *A. sinensis* also occurs in these countries, but the centre of its range is more to the northeast towards China and it does not appear to have penetrated further into the archipelago than the Malay Peninsula and Sumatra. Towards the limits of the Oriental region in northwest India and central and northern China, the southeast Asian species of *hyrcanus* are few or absent, and are replaced by other forms of *hyrcanus* of palaearctic type. In China, the name *sinensis* probably covers two overlapping forms, a southerly oriental one presumed to be Wiedemann's type form which lays a wide decked egg, and a northerly palaearctic form laying a narrow decked egg.

Species of which sufficient numbers have been seen from different countries show geographical variation. The effects of this variation are most marked in the relatively isolated Philippine Islands, where out of three forms occurring there, one, *pseudosinensis*, is regarded as a species peculiar to the Philippines, though clearly derived from the same stock as the widespread *nigerrimus* which does not occur there. A second, *lesteri*, is probably a subspecies of the form of *lesteri* found in Borneo and the Malay Peninsula. The group thus seems to conform to the classical pattern of speciation by geographical isolation. Much of the evolution and distribution of the species probably took place in pleistocene times.

Broadly speaking *A. 'hyrcanus'* can be described as a swamp breeder, with a preference for animal blood. But it is evident that the individual species show important differences in their biology. Some breed in sunlit waters and their larvae are often green; others seem to prefer shaded places, though not in jungle, and their larvae are usually dark coloured. *A. sinensis* and *lesteri* at least, can breed in moderately saline waters, sometimes in company with *A. sundaicus* and *baezai*.

It appears that most of the species will bite man freely out of doors shortly after dark, but are reluctant to enter rooms in search of human blood. However, the

degree of reluctance to bite man probably varies to an important extent between different species. All species can usually be captured at night at cattle sheds. The adults do not often rest indoors by day, and the outdoor resting places of *indiensis* and *lesteri* are described.

In southeast Asia, though '*hyrcanus*' is not one of the principal mosquito vectors of malaria or filariasis, it has been found infected on a number of occasions. In most cases the exact species cannot be determined now, but where this is possible, it has been *A. nigerrimus* Giles in every instance. Venhuis (1939) showed that his *A. hyrcanus* var. X (= *nigerrimus*) was a vector of malaria in Celebes and Java, and Kariadi (1941) implicated it in the transmission of malayan filariasis in southeast Borneo. In Malaya, examination of preserved specimens showed that *nigerrimus* was the principal or only vector during an outbreak of malaria at Kuala Lumpur which Hodgkin (1933) had shown to be due to *hyrcanus*, and recently *nigerrimus* has been found to play a part in the transmission of malayan filariasis in Kedah and Province Wellesley. Experimental infections with *Wuchereria malayi*, the worm which causes malayan filariasis, showed that *nigerrimus* was less readily infected than the principal vectors (*Mansonia* spp.), and some other members of the *hyrcanus* group, but that it bit the human carrier more readily than the latter. *A. sinensis*, though probably harmless in Malaya, is a vector of malaria and bancroftian filariasis in parts of China, but the exact identification of the vector is now in doubt, since there appear to be at least two forms in China included under the name *sinensis*.

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References.

- D'ABRERA, V. St. E. (1944). The eggs of the Ceylon Anopheline mosquitoes.—
J. Malar. Inst. India, **5**, pp. 337-359.

- BAISAS, F. E. (1931). The *barbirostris-hyrcanus* group of the Philippine *Anopheles*.—Philipp. J. Sci., **44**, pp. 425–448.
- BAISAS, F. E. & HU, S. M. K. (1936). *Anopheles hyrcanus* var. *sinensis* of the Philippines and certain parts of China, with some comments on *Anopheles hyrcanus* var. *nigerrimus* of the Philippines.—Mon. Bull. Bur. Hlth Philipp., **16**, pp. 205–242.
- BARRAUD, P. J. & CHRISTOPHERS, S. R. (1931). On a collection of Anopheline and Culicine mosquitoes from Siam.—Rec. Malar. Surv. India, **2**, pp. 269–285.
- BATES, M., BEKLEMISHEV, W. N. & LA FACE, L. (1949). Anophelines of the Palearctic region.—In Boyd, M. F. Ed. Malariology, **1**, pp. 419–442. Philadelphia, Pa., Saunders.
- BENTLEY, A. (1902). Anopheles mosquitoes in Tezpur, Assam.—Indian med. Gaz., **37**, pp. 15–16.
- BONNE-WEPSTER, J. (1951). *Anopheles venhuisi* n. sp.—Docum. neerl. indones. Morb. trop., **3**, p. 284.
- BRUG, S. L. & BONNE-WEPSTER, J. (1947). The geographical distribution of the mosquitoes of the Malay Archipelago.—Chron. nat., **103**, 19 pp.
- CHOW, C. Y. (1950). Mosquito studies in China, past and present.—Mosq. News, **10**, pp. 134–137.
- CHRISTOPHERS, S. R. (1924). Provisional list and reference catalogue of the Anophelini.—Indian med. Res. Mem., **3**, 105 pp.
- CHRISTOPHERS, S. R. (1933). The fauna of British India. Diptera, Vol. IV. Family Culicidae. Tribe Anophelini.—371 pp. London.
- CHRISTOPHERS, S. R. & BARRAUD, P. J. (1931). The eggs of Indian *Anopheles*, with descriptions of the hitherto undescribed eggs of a number of species.—Rec. Malar. Surv. India, **2**, pp. 61–192.
- CHRISTOPHERS, S. R. & CHAND, K. (1915). Notes on some Anophelines from Arabia and Mesopotamia.—Indian J. med. Res., **3**, pp. 180–200.
- COLLESS, D. H. (1948). The Anopheline mosquitoes of north-west Borneo.—Proc. Linn. Soc. N.S.W., **73**, pp. 71–119.
- COVELL, G. (1944). Notes on the distribution, breeding places, adult habits, and relation to malaria of the Anopheline mosquitoes of India and the Far East.—J. Malar. Inst. India, **5**, pp. 399–434.
- CRAWFORD, R. (1938). Some Anopheline pupae of Malaya with a note on pupal structure.—110 pp. Singapore, Govt. S.S. & Malar. Adv. Bd F.M.S.
- DOBBY, E. G. H. (1950). Southeast Asia. London.
- EDWARDS, F. W. (1921). A revision of the mosquitos of the Palaearctic region.—Bull. ent. Res., **18**, pp. 263–351.
- EDWARDS, F. W. (1929). Mosquito notes. VIII.—*Ibid.*, **20**, pp. 321–343.
- EDWARDS, F. W. (1932). Diptera. Fam. Culicidae.—Genera Insect., **184**, 245 pp. Brussels.
- EVANS, A. M. (1938). Mosquitoes of the Ethiopian region. II. Anophelini.—404 pp. London, Brit. Mus. (Nat. Hist.).
- FENG, L. C. (1938). A critical review of the literature regarding the records of mosquitoes in China.—Peking nat. Hist. Bull., **12**, pp. 169–181, 285–318.
- FOWLER, H. W. (1949). A dictionary of modern English usage.—London.

- GATER, B. A. R. (1933). Notes on Malayan mosquitoes, I. The genus *Anopheles*.—Malay. med. J., **8**, pp. 39-42.
- GATER, B. A. R. (1935). Aids to the identification of Anopheline imagines in Malaya.—242 pp. Singapore, Govt. S.S. & Malar. Adv. Bd F.M.S.
- GILES, G. M. (1900). A handbook of the gnats or mosquitoes.—374 pp. London.
- GILES, G. M. (1902). *Ibid.* 2nd edn., 530 pp.
- GILES, G. M. (1904). A revision of the Anophelinae.—48 pp. London.
- VAN HELL, J. C. (1950). De betekenis van *A. (A.) hyrcanus* var. X als malaria-overbrenger op Zuid-Celebes.—Med. Maandbl., **3**, pp. 557-567. In English in Docum. neerl. indones. Morb. trop., **3**, pp. 373-380.
- HO, CH'U. (1938). On a collection of Anopheline mosquitoes from the island of Hainan.—Ann. trop. Med. Parasit., **32**, pp. 387-411.
- HODGKIN, E. P. (1933). *Anopheles hyrcanus* Pallas as a malaria carrier in Malaya.—Bull. Inst. med. Res. F.M.S., no. 1 of 1932, pp. 1-6.
- HODGKIN, E. P. (1937). Division of Entomology.—Rep. Inst. med. Res. F.M.S., 1936, pp. 79-92.
- HODGKIN, E. P. (1939). Division of Entomology.—*Ibid.*, 1938, pp. 64-79.
- HODGKIN, E. P. (1940). The breeding of certain species of *Anopheles* in saline waters.—Trans Far-East Ass. trop. Med., 10th Congr., **2**, pp. 839-871.
- HODGKIN, E. P. & RAJAMONEY, P. D. (1933). A descriptive and biological note on the Malayan varieties of *Anopheles hyrcanus* Pallas.—Bull. Inst. med. Res. F.M.S., no. 1 of 1933, pp. 7-18.
- INSTITUTE FOR MEDICAL RESEARCH, MALAYA. (1950). Division of Entomology.—Rep. Inst. med. Res. Malaya, 1949, pp. 25-32.
- INSTITUTE FOR MEDICAL RESEARCH, MALAYA. (1951). Division of Entomology.—*Ibid.*, 1950, pp. 14-20.
- JAMES, S. P. & LISTON, W. G. (1904). A monograph of the Anopheline mosquitoes of India. Calcutta.
- JAMES, S. P. & LISTON, W. G. (1911). *Ibid.*, 2nd edn., 128 pp.
- JAMES, S. P. & STANTON, A. T. (1912). Revision of the names of Malayan Anophelines.—Paludism, **5**, pp. 59-63.
- KARIADI, —. (1941). *A. hyrcanus* X en filariasis malayi te Martapoera.—Geneesk. Tijdschr. Ned.-Ind., **81**, pp. 107-118.
- KNIGHT, K. L. & CHAMBERLAIN, R. W. (1948). A new nomenclature for the chaetotaxy of the mosquito pupa, based on a comparative study of the genera (Diptera : Culicidae).—Proc. helminth. Soc. Wash., **15**, pp. 1-10.
- LAMBORN, W. A. (1921). Federated Malay States Malaria Bureau Report, 1920.—Suppl. F.M.S. Govt. Gaz., 4th Nov. 1921, pp. 8-13.
- LAMBORN, W. A. (1922a). The mosquitos of some ports of China and Japan.—Bull. ent. Res., **12**, pp. 401-409.
- LAMBORN, W. A. (1922b). The bionomics of some Malayan Anophelines.—Bull. ent. Res., **13**, pp. 129-149.
- LAVERAN, A. (1902). Sur des culicides du Cambodge.—C.R. Soc. Biol., Paris, **54**, pp. 906-908.
- LEICESTER, G. F. (1908). The Culicidae of Malaya.—Stud. Inst. med. Res. F.M.S., **3**, no. 3, pp. 18-261.

- McARTHUR, J. (1950). Malaria and its vectors in Borneo.—*Indian J. Malariol.*, **4**, pp. 1–90.
- MAYR, E. (1944). Wallace's line in the light of recent zoogeographic studies.—*Quart. Rev. Biol.*, **19**, pp. 1–14; also in *Science and Scientists in the Netherlands Indies*. New York, 1945.
- MAYR, E. (1947). Systematics and the origin of species.—3rd edn. New York.
- OTSURU, M. & MIYAGAWA, M. (1950). The eggs of Japanese *Anopheles*.—*Jap. J. sanit. Zool.*, **1**, pp. 3–4 (Abstract only seen).
- PITTENDRIGH, C. S. (1950). The ecotype specialization of *Anopheles homunculus*, and its relation to competition with *A. bellator*.—*Evolution*, **4**, pp. 64–78.
- POYNTON, J. O. & HODGKIN, E. P. (1938). Endemic filariasis in the Federated Malaya States.—*Bull. Inst. med. Res. F.M.S.*, no. 1 of 1938, 67 pp.
- REID, J. A. (1947). Type specimens of Culicidae described by Laveran. (Diptera : Culicidae).—*Proc. R. ent. Soc. Lond.*, (B) **16**, pp. 86–91.
- REID, J. A. (1949). A preliminary account of the forms of *Anopheles leucosphyrus* Dönitz (Diptera : Culicidae).—*Proc. R. ent. Soc. Lond.*, (B) **18**, pp. 42–53.
- REID, J. A. (1950). Some new records of Anopheline mosquitoes from the Malay Peninsula with remarks on geographical distribution.—*Bull. Raffles Mus.*, **21**, pp. 48–58.
- REID, J. A. & HODGKIN, E. P. (1950). The *Anopheles umbrosus* group (Diptera : Culicidae).—*Trans. R. ent. Soc. Lond.*, **101**, pp. 281–334.
- RUSSELL, P. F. & BAISAS, F. E. (1934). A practical illustrated key to the larvae of Philippine *Anopheles*.—*Philipp. J. Sci.*, **55**, pp. 307–336.
- RUSSELL, P. F. & BAISAS, F. E. (1936). A practical illustrated key to adults of Philippine *Anopheles*.—*Philipp. J. Sci.*, **58**, pp. 15–64.
- SCRIVENOR, J. B. & others. (1943). A discussion on the biogeographic division of the Indo-Australian archipelago, with criticism of the Wallace and Weber lines . . .—*Proc. Linn. Soc. Lond.*, **154**, pp. 120–165.
- VAN SOMEREN, E. C. C. (1947). The description of a new *Anopheles* of the *Myzorhynchus* series from Madagascar, with notes on its systematic position in relation to the Ethiopian species of this group.—*E. Afr. med. J.*, **24**, pp. 42–46.
- STANTON, A. T. (1915). Notes on Sumatran Culicidae.—*Indian J. med. Res.*, **3**, pp. 251–258.
- STANTON, A. T. & HACKER, H. P. (1917). The *Anopheles* of Malaya. III. A new variety of *A. albotaeniatus*, Theo.—*Bull. ent. Res.*, **7**, pp. 273–275.
- STOKER, W. J. (1931). *Anopheles montanus* (*Anopheles albotaeniatus* var. *montanus* Stanton and Hacker, 1917).—*Meded. Dienst Volksgezondh. Ned.-Ind.*, **20**, pp. 129–132.
- SWEET, W. C., FENG, L. C., CHOW, C. Y. & HSU, S. C. (1942). Anophelines of southwestern Yunnan and their relation to malaria.—*J. nat. Malar. Soc.*, **1**, pp. 25–32.
- SWELLENGREBEL, N. H. (1914). Een nieuwe anopheline voor Deli, *Myzorhynchus argyropus* n. sp.—*Geneesk. Tijdschr. Ned.-Ind.*, **54**, p. 334.
- SWELLENGREBEL, N. H. (1921). De Anophelinen van Nederlandsch Oost-Indië. 2nd edn.—*Meded. kolon. Inst. Amst.*, (Trop. Hyg.) no. 10, 155 pp.
- SWELLENGREBEL, N. H. & RODENWALDT, E. (1932). Die Anophelen von Niederländisch-Ostindien.—242 pp. Jena, Fischer.

- THEOBALD, F. V. (1901). A monograph of the Culicidae, **1**. London, Brit. Mus. (Nat. Hist.).
- THEOBALD, F. V. (1903). *Ibid.*, **3**.
- THEOBALD, F. V. (1907). *Ibid.*, **4**.
- THOMSON, R. C. M. (1940). Studies on the behaviour of *Anopheles minimus*. Part III. The influence of water temperature on the choice and suitability of the breeding place.—J. Malar. Inst. India, **3**, pp. 323–348.
- THOMSON, R. C. M. (1951). Studies on salt-water and fresh-water *Anopheles gambiae* on the East African coast.—Bull. ent. Res., **41**, pp. 487–502.
- VENHUIS, W. G. (1939). The *hyrcanus* problem in the Netherlands East Indies, with description of a widespread malaria carrying variety: *An. hyrcanus* X. (First communication).—Meded. Dienst Volksgezondh. Ned.-Ind., **28**, pp. 376–389.
- WALCH, E. W. (1930). The larva of *Anopheles peditaeniatus* (Leicester).—*Ibid.*, **19**, pp. 44–45.
- WALCH, E. W. & WALCH-SORGDRAGER, G. B. (1935). The eggs of some Netherlands-Indian Anophelines.—Trans. Far East Ass. trop. Med., 9th Cong., pp. 65–81. In Dutch in Geneesk. Tijdschr. Ned.-Ind., **75**, pp. 1700–1730, with plates.
- WHARTON, R. H. (1950). Daytime resting places of *Anopheles maculatus* and other Anophelines in Malaya, with results of precipitin tests.—Med. J. Malaya, **4**, pp. 260–271.
- WIEDEMANN, C. R. W. (1828). Aussereuropäische zweiflügelige Insekten, **1**, p. 547. Hamm.
- WIGGLESWORTH, V. B. (1942). The principles of insect physiology. 2nd edn. London, Methuen.
- WILSON, T. & REID, J. A. (1951). Filariasis.—Stud. Inst. med. Res. Malaya, **25**, pp. 209–227.
- YAMADA, M. (1927). A new species of *Anopheles* in Chosen (Korea).—Keijo J. Med., **3**, pp. 237–255.
- YAMADA, S. (1924). A revision of the adult Anopheline mosquitoes of Japan. Part 1.—Sci. Rep. Inst. infect. Dis. Tokyo Univ., 1924, **3**, pp. 215–241.
- YAO, Y. T. & WU, C. C. (1935). One year's observation of *Anopheles hyrcanus* var. *sinensis* in Nanking, 1933.—Trans. Far-East Ass. trop. Med., 9th Congr., **2**, pp. 3–26.
- YAO, Y. T. & WU, C. C. (1936). Some abnormalities of the morphology of the male hypopygia of *Anopheles hyrcanus* var. *sinensis* Wied., in Nanking.—Peking nat. Hist. Bull., **11**, pp. 27–34.
- ZEUNER, F. E. (1941). Geology, climate and faunal distribution in the Malaya archipelago.—Proc. R. ent. Soc. Lond., (A) **16**, pp. 117–123.

Statistical Data.

This section contains a rather unwieldy but necessary mass of figures, inserted at this point as being the least inconvenient place. Table IV compares the larval and pupal chaetotaxy of *pseudosinensis*, *sinensis* and *nigerrimus*; my views on the interrelation of these three forms is based in part on these figures. Table V contributes to the view that Malayan and Philippine *lesteri* are forms of one species. Table VI sets out the larval chaetotaxy of Malayan forms and is part of the descriptions, necessary to any further work on the group. Tables VII to IX also form part of the descriptions.

TABLE IV.
To compare the larval and pupal chaetotaxy of *pseudosinensis* with *nigerrimus* and *sinensis*.

Species	Larval head				Larval thorax				Larval abdomen				Pupa	
	Hair No. 2	Hair No. 4	Hair No. 8	Hair No. 9	Hair No. 10	I.1	I.2	I.13	II.5	III.6	VI.5	VI.9	Pecten long teeth	V.5
1. <i>pseudosinensis</i> ...	iac 1-8 1-8	pc 1-5 3	5-12 8	3-8 6	6-17 9-6	1-4 1	8-14 11	6-13 9	2-5 3	9-20 13-9	4-5	3-4	6	4-11
2. <i>nigerrimus</i> ...	1-2 1-1	2-7 4	12-24 17	8-14 11	10-20 11-9	1-4 3	6-13 10	3-8 7	5-10 7	12-20 16-6*	2-5*	4*	7*	30-60
3. <i>sinensis</i> ... (Hong Kong)	1-2 1-0	2-6 3*	5-11 8*	4-9 6*	2-11 4-8	1-3 1*	8-13 11*	6-11 8	2-6 3*	15-23 18-3				
4. <i>sinensis</i> ... (Malaya)	1-4 1-3*	3-8 5	8-13 11	7-11 9	7-14 10-0*	1-5 3	9-16 12	6-12 9*	4-8 6	17-29 23-8	6-11	6-10	8	9-24*

* Denotes the number closest to that of *pseudosinensis*. Figures in bold type are the average or commonest number of branches. The figures for *pseudosinensis* and *sinensis* (Hong Kong) are from Baisas and Hu, except those for *pseudosinensis* in columns 2, 3 and 4 of larval abdomen.

TABLE V.
To compare the larval and pupal chaetotaxy of typical *lestevi* from the Philippines with Malayan specimens.

Species	Larval head				Larval thorax				Larval abdomen				Pupa	
	Hair No. 2	Hair No. 4	Hair No. 8	Hair No. 9	Hair No. 10	I.1	I.2	I.13	II.5	III.6	II.5	VI.5	VI.9	Pecten long teeth
<i>lestevi</i>														
1. Ex Philippines ...	1-1 1	2-5 3	5-12 9	7-10 9	8-13 10	1-3 2	7-14 9	5-8 6	3-6 4	13-23 18	6-11	5-7	5-6	8
2. Ex Malaya ...	1-1 1	2-5 3	5-11 9	6-11 8	7-15 9	1-3 2	8-14 9	5-8 6	3-8 5	17-23 20	6-10	5-8	4-7	8

The figures for *lestevi* from the Philippines are from Baisas and Hu, except for those in columns 2 to 5 of larval abdomen, and those for the pupa; the latter combines the figures of Baisas and Hu with lower counts obtained on 12 of Rozeboom's specimens from Samar, P.I. Figures in bold type are the commonest number.

TABLE VI.

Larval chaetotaxy. Malayan specimens.

Setae (Figs. in brackets approximate number examined.)	<i>sinensis</i> 1.	<i>nigerrimus</i> 2.	<i>indiensis</i> 3.	<i>peditaen- ratus</i> 4.	<i>argyropus</i> 5.	<i>lesteri</i> 6.	<i>crawfordi</i> 7.
HEAD							
2 (20-48) ...	1-4	1-2	1	1	1	1	1-2
3 (5) ...	65-80	70-85	70-80	40-70	70-90	43-70	50-55
4 (40) ...	3-8	2-7	1-6	3-7	2-6	2-5	2-6
5 (10) ...	17-24	11-18	12-18	14-18	12-16	10-16	13-16
6 (10) ...	17-20	11-17	11-18	13-20	12-20	13-15	14-17
7 (10) ...	17-22	14-22	14-19	16-19	14-18	13-18	14-17
8 (40) ...	8-13	12-24	11-17	6-9	13-22	5-11	6-11
9 (40) ...	7-11	8-14	6-13	4-7	9-16	6-11	5-10
10 (40) ...	7-14	10-20	9-15	3-12	11-20	7-15	9-15
11 (5-25) ...	6-10	6-10	7-9	4-9	6-11	5-11	6-10
14 (5-10) ...	3-5	3-4	2-4	3-5	2-3	3-4	2-4
15 (10-25) ...	3-7	3-6	2-4	2-5	2-5	2-4	2-3
20 (5-20) ...	10-20	5-10	4-11	4-8	4-6	5-8	3-8
PROTHORAX							
1 (20-40) ...	1-5	1-4	1-4	1-3	2-3	1-3	1-8
2 (20-40) ...	9-16	6-13	11-19	10-17	8-13	8-14	6-12
4 (15-20) ...	11-19	12-17	12-16	10-16	12-16	11-18	13-20
5 (10-20) ...	23-34	21-34	20-31	18-27	18-26	17-27	21-25
7 (10-20) ...	22-34	19-24	21-29	21-26	20-24	18-25	21-26
8 (10-20) ...	22-30	18-23	17-26	17-24	18-22	16-23	19-24
13 (40) ...	6-12	3-8	5-10	3-6	6-11	5-8	6-10
14 (40) ...	11-17	11-20	10-17	8-16	9-14	10-16	10-17
MESOTHORAX							
1 (10-20) ...	24-31	21-27	19-32	21-27	21-25	22-30	22-29
5 (40) ...	4-8	5-10	6-10	4-10	6-12	3-8	4-8
6 (10-20) ...	4-6	3-6	3-5	3-5	4-5	3-5	3-5
7 (20-40) ...	2-4	2-5	3-5	2-4	4-11	2-4	2-4
8 (15-20) ...	14-22	11-18	14-20	11-18	12-17	10-15	12-15
13 (10) ...	13-24	12-25	15-18	10-15	20-30	16-20	12-17
14 (10-20) ...	7-14	12-18	6-15	6-11	7-16	5-10	8-10
METATHORAX							
2 (20) ...	3-7	2-5	3-6	3-7	3-6	3-6	3-8
5 (15-20) ...	28-37	25-36	22-28	25-34	22-31	24-31	21-33
6 (20) ...	3-4	2-5	2-5	2-5	3-7	2-4	3-7
7 (15) ...	23-31	17-27	20-25	19-30	18-24	19-26	18-23
8 (10-20) ...	26-31	21-33	21-28	22-29	20-24	19-26	21-28
13 (20) ...	3	1-3	2-3	1-4	2-4	2-3	3-4
ABDOMEN I							
2 (20) ...	4-8	5-10	7-10	6-8	5-8	4-9	6-10
4 (20) ...	8-15	4-9	6-11	6-11	5-9	8-16	8-12
5 (40) ...	4-8	3-7	4-6	4-8	3-6	3-5	3-7
6 (20) ...	21-28	15-20	16-22	18-25	16-19	17-21	16-21
7 (20) ...	18-25	13-18	14-19	16-24	13-19	12-19	14-19
9 (40) ...	5-11	3-5	3-7	4-7	4-6	4-7	5-8
11 (20) ...	3-5	2-3	2-3	3-4	2-3	2-4	4-6
13 (10-20) ...	10-17	8-15	10-15	8-13	12-18	10-15	10-15
ABDOMEN II							
2 (20) ...	6-12	6-12	8-12	5-9	6-9	6-10	8-15
3 (20) ...	5-12	3-7	4-10	4-9	4-6	6-9	6-10
5 (40) ...	9-20	4-11	8-17	9-15	9-15	6-10	10-18
6 (20) ...	23-33	20-26	18-25	22-29	18-22	18-26	18-23
7 (20) ...	21-28	16-23	15-21	21-27	17-23	17-24	16-24
9 (40) ...	8-16	5-9	5-12	9-16	5-10	5-10	7-13
10 (20) ...	3-5	2-3	2-4	2-3	2-3	2-4	2-3
13 (10-20) ...	9-14	9-15	10-15	8-12	12-17	10-13	7-14

TABLE VI (continued).
Larval chaetotaxy. Malayan specimens.

Setae (Figs. in brackets approximate number examined.)	<i>sinensis</i> 1.	<i>nigerri- mus</i> 2.	<i>indiensis</i> 3.	<i>peditaen- iatus</i> 4.	<i>argyropus</i> 5.	<i>lestevi</i> 6.	<i>crawfordi</i> 7.
ABDOMEN III							
1 (20) ...	16-27	17-24	16-25	16-25	17-25	15-21	16-22
2 (20) ...	5-10	2-6	5-9	3-7	4-8	5-8	6-9
3 (20) ...	3-7	2-4	2-6	2-6	2-5	4-7	4-6
5 (40) ...	8-17	4-8	8-17	6-14	7-12	7-11	9-14
6 (20) ...	17-29	12-20	15-20	16-22	14-20	17-23	15-21
7 (20) ...	4-8	1-3	2-4	3-6	2-5	4-5	4-8
9 (40) ...	8-14	5-10	10-16	9-18	6-13	5-10	5-11
13 (10-20) ...	8-13	6-11	10-14	8-13	9-18	9-12	8-14
ABDOMEN IV							
1 (20) ...	17-24	14-26	16-24	13-25	15-25	18-23	18-23
3 (20) ...	4-6	2-5	3-5	3-6	3-4	4-7	5-8
4 (20) ...	2-6	2-4	3-4	2-3	2-3	2-4	2-4
5 (40) ...	3-7	2-4	5-9	3-5	4-10	3-6	4-8
7 (20) ...	4-8	2-4	3-6	4-5	3-6	3-5	4-6
9 (40) ...	10-16	5-9	8-15	9-14	8-11	6-13	6-11
13 (20-40) ...	3-6	3-6	6-12	3-6	7-10	4-9	4-7
ABDOMEN V							
1 (20) ...	16-25	16-25	14-23	15-25	15-19	15-23	19-24
3 (20) ...	4-7	2-4	3-6	3-5	3-4	4-6	4-6
5 (40) ...	4-10	2-4	5-9	4-7	4-5	4-6	5-9
9 (40) ...	9-15	4-8	7-12	8-12	8-10	6-10	6-11
13 (20) ...	3-5	3-4	3-4	3-4	4	3-5	3-5
ABDOMEN VI							
1 (20) ...	16-24	16-24	16-22	15-24	14-18	16-24	18-22
3 (20) ...	3-10	3-7	4-8	4-7	3-5	3-6	4-11
5 (40) ...	6-11	2-5	5-9	5-8	5-6	5-8	6-10
9 (40) ...	6-10	2-4	5-9	5-9	4-5	4-7	5-8
13 (20) ...	9-18	7-14	10-15	10-20	13-14	10-18	9-16
ABDOMEN VII							
1 (20) ...	17-26	14-20	15-22	16-24	17-19	17-24	17-23
2 (20) ...	6-14	4-10	5-10	5-10	6-7	5-8	6-10
3 (20) ...	2-5	2-4	2-5	2-5	3-4	2-6	3-4
5 (40) ...	5-10	3-6	5-10	6-9	4-6	4-8	5-9
9 (40) ...	4-7	2-4	3-8	4-6	3	3-5	4-7
13 (20) ...	3-5	2-5	2-4	3-4	3-4	3-4	2-3
ABDOMEN VIII							
5 (10) ...	2-3	1-3	1-4	1-2	1-2	1-2	2-3
9 (40) ...	5-9	4-9	5-11	8-12	7-8	5-9	7-12
13 (20) ...	4-6	3-5	3-5	3-5	4	3-5	4-5
ABDOMEN IX							
6 (20) ...	7-10	5-7	5-8	5-9	5-6	3-8	4-7
8 (20) ...	5-9	5-10	4-7	5-6	4-5	4-7	3-7
9 (40) ...	4-7	3-5	3-5	4-8	4	3-5	3-5

TABLE VII.
Some measurements of the pupae.

Measurement	<i>sinensis</i>	<i>nigerri- mus</i>	<i>indiensis</i>	<i>peditaen- iatus</i>	<i>argyropus</i>	<i>lesteri</i>	<i>crawfordi</i>
<i>Abdomen segment V</i>							
branches on hair 2	3-13	7-20	9-28	2-6	17-40	7-13	7-19
" " " 5	9-24	40-60	30-50	14-28	>50	12-20	30-40
<i>Paddle</i>							
length mm.80	.72	.64	.75	.68	.66	.76
width /length79	.76	.73	.71	.76	.74	.76
length of refractile border /total length.							
Range59-.70	.63-.71	.67-.80	.83-.93	.81-.91	.52-.64	.60-.70
Mean65	.67	.75	.88	.87	.58	.65

The figures in this table are based on measurements of not less than five specimens of each species; those for hairs 2 and 5 include Crawford's data.

TABLE VIII.
Some measurements of the eggs.

Measurement	<i>sinensis</i> 1.	<i>nigerri- mus</i> 2.	<i>indiensis</i> 3.	<i>peditaen- iatus</i> 4.	<i>argyropus</i> 5.	<i>lesteri</i> 6.	<i>crawfordi</i> 7.
Length mm. (10)52	.53	.52	.57	.53	.55	.56
Deck width mm. (10)	.054	.019	.033	.020	.008	.012	.009
Deck width /length	.10	.03	.06	.03	.01	.02	.01
Deck width / Total width (approx- imate) ...	1/3	1/7	1/6	1/6	<1/20	<1/10	<1/20
No. float ribs (5) ...	30-35	35-40	25-30	25-30	30-35	24-28	27-30

Figures in brackets in the first column are the numbers of specimens measured for each species.

TABLE IX.
Thoracic chaetotaxy of adult females.

Setae	<i>sinensis</i>	<i>nigerrimus</i>	<i>indiensis</i>	<i>peditaen- iatus</i>	<i>argyropus</i>	<i>lesteri</i>	<i>crawfordi</i>
	1.	2.	3.	4.	5.	6.	7.
Propleural ...			See Table 7				
Upper sternopleural—							
Range ...	5-6	3-4	3-4	2-3	3-4	2-5	3-5
Mean ...	5.2	3.4	3.6	3.0	3.2	3.6	4.2
Lower sternopleural—							
Range ...	5-9	4-7	4-7	4-6	4-6	4-8	3-5
Mean ...	6.4	5.4	5.2	4.6	4.6	5.6	3.8
Spiracular—							
Range ...	4-5	2-3	2-3	2-4	2-4	2-5	3-4
Mean ...	4.6	2.8	2.8	2.8	2.6	3.6	3.2
Prealar—							
Range ...	8-12	5-9	8-10	6-9	5	6-7	6-9
Mean ...	10.4	7.2	8.4	7.2	5	6.7	6.8
Subalar—							
Range ...	5-10	4-6	5-9	4-9	3-7	6-7	6-8
Mean ...	7.6	5.4	6.4	6.2	4.8	6.6	6.8
Upper mid-coxal—							
Range ...	4-5	1-3	0-1	3-5	3-4	3-4	3-4
Mean ...	4.4	2.2	1	3.8	3.8	3.8	3.6

Samples of five for each species, except upper mid-coxals of *indiensis* (20).

Tables X-XIII contain the statistical backing for much of the preceding section on interrelations. These Tables also show that in addition to the few clear cut characters that can be used in keys, sibling species of *Anopheles* may show statistically significant differences in a wide range of characters affecting the size of parts. Inspection may suggest that a small difference of this sort exists between two forms, but measurement is needed to confirm or disprove it. However, measurements on as few as 5 specimens (Table XIII) may reveal significant differences that might be of value for identification in any future studies.

TABLE X.

Number of observations, range, mean and standard deviation of various measurements on adults and larvae. See also Table III.

Measurements	1. <i>sinensis</i>	2. <i>nigerri- mus</i>	3. <i>indiensis</i>	4. <i>peditaen- iatus</i>	5. <i>argyropus</i>	6. <i>lesteri</i>	7. <i>crawfordi</i>
<i>Wing length, mm.</i> ¹							
50 observations—							
Range	3.00–4.33	3.06–4.26	2.86–4.00	2.94–3.86	3.14–3.94*	3.40–4.33	2.94–4.20
Mean	3.84	3.82	3.46	3.50	3.56	3.83	3.79
Standard deviation	.31	.22	.21	.21	.17	.21	.25
<i>Wing width, mm.</i> ¹							
50 observations—							
Range73–1.00	.77–.97	.70–.90	.67–.87	.67–.87*	.70–.93	.67–.93
Mean90	.87	.77	.78	.79	.83	.83
S.D.	0.062	.045	.046	.044	.036	.050	.057
<i>Propleural setae</i>							
50 observations—							
Range	4–10	5–10	2–5	2–6	3–7	3–6	2–5
Mean	6.9	7.5	3.7	4.1	5.0	3.9	2.9
S.D.	1.29	0.97	0.83	0.93	0.96	0.75	0.60
<i>Pecten long spines</i> ²							
Observations ...	54	45	46	28	41	34	24
Range	7–9	6–7	5–7	7–9	6–8	7–10	7–8
Mean	7.8	6.6	5.9	8.0	7.1	8.1	7.5
S.D.54	.49	.51	.51	.52	.69	.51
<i>Palmate hairs IV or V, diameter, mm.</i>							
20 observations—							
Range19–.22	.18–.21	.15–.17	.17–.20	.16–.19	.13–.18	.12–.16
Mean20	.19	.16	.18	.18	.16	.14
S.D.012	.012	.006	.007	.010	.006	.013
<i>Tergal plate VIII, length, mm.</i>							
20 observations—							
Range16–.19	.16–.21	.15–.19	.16–.21	.16–.19	.15–.21	.14–.19
Mean18	.17	.17	.18	.18	.19	.16
S.D.009	.013	.013	.014	.008	.016	.015
<i>Tergal plate VIII, width, mm.</i>							
20 observations—							
Range26–.34	.24–.32	.19–.27	.22–.30	.22–.28	.22–.29	.19–.26
Mean30	.28	.24	.25	.25	.26	.23
S.D.021	.022	.021	.023	.017	.019	.022
<i>Larval antenna, shaft length, mm.</i>							
20 observations—							
Range25–.30	.22–.26	.22–.26	.22–.25	.22–.24	.22–.26	.22–.25
Mean28	.24	.24	.24	.23	.24	.23
S.D.012	.009	.012	.009	.009	.011	.008
<i>Larval antenna, shaft width, mm.</i>							
20 observations—							
Range05–.06	.04–.06	.04–.05	.04–.05	.04–.05	.04–.05	.04–.05
Mean05	.05	.05	.04	.04	.04	.05
S.D.002	.004	.003	.003	.001	.002	.003

¹Exclusive of the fringe. ²The first or most dorsal spine has been counted as a long one if it is longer than the majority of the small teeth.

*Only 32 observations.

TABLE XI.

To compare the coefficients of variability (c.v.) of four characters. See Table VII.

Character	1. <i>sinensis</i>	2. <i>nigerrimus</i>	3. <i>indiensis</i>	4. <i>peditaeniatius</i>	5. <i>argyropus</i>	6. <i>lesteri</i>	7. <i>crawfordi</i>
Wing length ...	8.0	5.8	6.0	5.9	4.7	5.4	6.5
Propleural setae ...	18.7	12.9	22.1	22.7	19.1	19.2	20.6
Pecten long spines...	6.9	7.4	8.6	6.4	7.4	8.6	6.8
Tergal plate VIII, width ...	7.0	7.7	8.4	9.1	6.7	7.4	9.5
Mean c.v. ...	10.15	8.45	8.77	11.02	9.47	10.15	10.85

The coefficient of variability is the standard deviation of a mean expressed as a percentage of that mean. The table shows that the number of propleural setae is a more variable character than the others.

TABLE XII.

To show the significance or otherwise of the differences between the means of three characters. The figures in the columns are the differences between the means divided by the standard errors of those differences. A figure of two or more is regarded as significant (difference at least twice the standard error). See Table VII.

Pairs of species	Wing length	Propleural setae	Pecten long spines
<i>sinensis</i> — <i>nigerrimus</i>	0.52	2.62	11.68
<i>indiensis</i>	7.36	14.66	18.27
<i>peditaeniatius</i>	6.49	12.53	1.08
<i>argyropus</i>	5.34	8.35	6.22
<i>lesteri</i>	0.20	14.20	1.61
<i>crawfordi</i>	0.91	19.86	2.95
<i>nigerrimus</i> — <i>indiensis</i>	8.36	20.91	6.77
<i>peditaeniatius</i>	7.26	17.94	11.13
<i>argyropus</i>	5.89	12.94	4.76
<i>lesteri</i>	0.46	20.69	10.29
<i>crawfordi</i>	0.48	28.43	6.58
<i>indiensis</i> — <i>peditaeniatius</i>	1.05	2.04	16.84
<i>argyropus</i>	2.36	7.15	11.07
<i>lesteri</i>	9.06	1.14	15.26
<i>crawfordi</i>	7.37	5.67	12.05
<i>peditaeniatius</i> — <i>argyropus</i>	1.48	4.87	6.47
<i>lesteri</i>	7.91	1.06	0.62
<i>crawfordi</i>	6.35	7.53	3.57
<i>argyropus</i> — <i>lesteri</i>	6.49	6.39	6.31
<i>crawfordi</i>	5.11	13.13	2.35
<i>lesteri</i> — <i>crawfordi</i>	0.88	7.35	3.80
Number of differences significant	13/21	19/21	18/21

Although judged by the coefficient of variability (see Table VIII) the number of propleural setae is a rather variable character, and in consequence is only of minor value for key purposes, the differences between the means of most of the pairs are highly significant.

There is not space to give the complete figures for other characters, but there are three which have been used in the keys and/or in classification (Table III) which should be briefly mentioned. PALMATE HAIRS (data in Table VII). These are bigger in *sinensis* and *nigerrimus* than in the other species, and the larger differences which have been tested are significant; *sinensis-crawfordi*, difference between the means 15.17 times the standard error of the difference, *nigerrimus-lesteri* 10.00. TERGAL PLATE VIII, LENGTH/WIDTH (means in Table III). Relatively wider in *sinensis* and *nigerrimus* than in the other species; *sinensis*, standard deviation of the mean 0.043, *nigerrimus* 0.032, *argyropus* 0.035, *crawfordi* 0.056; significance of differences, *sinensis-argyropus* 9.58, *nigerrimus-crawfordi* 6.48. WING, WIDTH/LENGTH (means in Table III). Wings relatively broader in *sinensis* and *nigerrimus*; *sinensis*, standard deviation of the mean 0.0087, *nigerrimus* 0.0077, *lesteri* 0.0066, *crawfordi* 0.0069; significance of differences, *sinensis-lesteri* 11.25, *nigerrimus-crawfordi* 6.44.

TABLE XIII.

Some differences in the dimensions of parts between *sinensis* and *lesteri*. Average size in millimetres of five specimens of each.

Species	Wing length	Fore femur	Fore tibia	Fore tarsi, one + two	Proboscis
<i>sinensis</i>	3.74	1.78	2.06	2.22	2.08
<i>lesteri</i>	3.63	1.85	2.24	2.47	1.95

It was noticed that the fore tarsi of *lesteri* seemed somewhat longer or more slender than those of *sinensis*. This was investigated by making a few measurements, summarised in the Table above. There was no time to measure more than five specimens.

If the length of the fore femur is expressed as a percentage of the total length, fore femur + tibia + tarsi 1 and 2, the results are :

sinensis 28.8 to 29.4 per cent., mean 29.10

lesteri 27.7 to 28.6 per cent., mean 28.22

The difference between these means is 0.88 per cent., and when examined by the "t" test for small samples, P is less than 0.01, so that the difference is significant. In other words, as suspected, the fore femur relative to the tarsi is proportionately shorter and the tarsi longer in *lesteri* than in *sinensis*. But if the examination is pursued, one sees that in terms of wing length, *lesteri* has a longer fore femur than *sinensis*; the ratios fore femur/wing being, *sinensis* 0.48, *lesteri* 0.51. We may say then that *lesteri* has a relatively longer fore leg, especially distally, than *sinensis*. The proboscis, however, in terms of wing length, is shorter in *lesteri* than *sinensis* (ratios proboscis/wing, *sinensis* 0.56, *lesteri* 0.54), and consequently the ratios fore femur/proboscis differ considerably (*sinensis* 0.86, *lesteri* 0.95).

A more thorough study of such differences in the relative sizes of parts would probably shed further light on the interrelations of the species, but it is very tedious work. One point has been noticed, however, and that is that the proboscis in members of the *umbrosus* group is usually shorter than the fore femur, in contrast to 'hyrcanus' and other species in the series *Myzorhynchus* in which it is longer. The ratios fore femur/proboscis for a number of species, determined on five or more specimens of each, were as follows.

Species					Ratio fore femur/proboscis
<i>brevirostris</i>	u	1.37
<i>brevipalpis</i>	u	1.08
<i>umbrosus</i>	u	1.07
<i>montanus</i>		1.04
<i>letifer</i>	u	1.02
<i>baezai</i>	u	1.01
<i>roperi</i>	u	0.97
<i>lesteri</i>		0.95
<i>separatus</i>	u	0.94
<i>albotaeniatus</i>		0.94
<i>argyropus</i>		0.88
<i>sinensis</i>		0.86
<i>indiensis</i>		0.86
<i>barbirostris</i>		0.85

u=*umbrosus* group (see Reid & Hodgkin, 1950).

A. roperi and *separatus*, which have the proboscis longer than the fore femur, though belonging to the *umbrosus* group because the larvae lack palmate hairs, also show other affinities with '*hyrcanus*' and its allies.

2 SOME NOTES ON *ANOPLONYX DESTRUCTOR* BENSON.

By Myles CROOKE.

Forestry Commission Research Station, Alice Holt Lodge, nr. Farnham.

E.M.A.

(PLATE I.)

A larch-feeding sawfly, which has become of increasing importance in British forests in recent years, has until now been identified and referred to as *Anoplonyx duplex* (Lep.) in a number of Forestry Commission publications (1950, 1951). It has nevertheless been known for some time that a number of differences, both morphological and biological existed between the *Anoplonyx* species common in Britain and that known as *duplex* in central Europe. Benson (1952) has recently reviewed the situation in detail after having examined material of this genus from the continent of Europe and from America. That author has shown that the species now common in Britain, and which is known to occur elsewhere only in Finland, is distinct from all other previously described species of *Anoplonyx* and he has designated it *A. destructor*. The situation is one of particular interest since it follows that, *Larix* being non-indigenous to Britain, the species must originally have been introduced into this country where it has developed into a pest of some note. Not only is it curious to observe that *A. destructor* has hitherto been unrecognised in its native habitat but also that nowhere in Europe has any member of the genus *Anoplonyx* occurred in sufficient numbers to warrant recognition as a forest pest.

For a number of years now the entomology staff of the Forestry Commission Research Station has been conducting an investigation into the biology, ecology, and forest status of the sawfly complex associated with larch crops in Britain. The



Fig. 1.—*Anoplonyx destructor* Benson, ♀ (× 9).

species studied include *Pristiphora erichsoni* (Hartig), *P. laricis* (Hartig), *P. wesmaeli* (Tischbein), *Pachynematus imperfectus* (Zaddach & Brischke), and *A. destructor* Benson. The present paper details what is at present known of the biology and forest status of *A. destructor* in Britain.

Life-cycle.

In the course of several years' study, during which large numbers of *A. destructor* have been handled, no males have been found and the species appears, therefore, to be entirely parthenogenetic. The females (fig. 1), which have been described in detail by Benson, are on the wing from the middle of April until early June. The eggs (Pl. I, fig. 1), which measure 1.25 mm. in length by 0.24 mm. in breadth, are deposited singly in the needles of the short shoots of larch. The ovipositing slit cut by the female is considerably longer than the length of the egg and typically causes a portion of the needle to become yellow and die. The egg stage lasts for 14–15 days and the resultant first-instar larva immediately commences feeding on the larch foliage (Pl. I, fig. 2). The larval feeding period has been completed in as short a time as 43 days under laboratory conditions with a mean temperature of 60°F. but more usually in nature extends throughout the whole of the summer and into the autumn, larvae being found still feeding as late as the end of September and early October. Four ecdyses occur during the feeding period after which the larvae descend from the foliage and spin their cocoons in the upper layers of the soil litter. These cocoons measure, on average, 8.1 mm. in length by 4.0 mm. in breadth. Pupation normally takes place in the following spring to give rise to the adults but is sometimes delayed until the second spring after spinning.

Larval Descriptions.

First Instar.—Length 2.07 mm. on hatching increasing to a maximum of 3.91 mm. Average width of head capsule 0.42 mm. (0.40 to 0.43 mm.). Head brown, with sparse, very fine, reddish setae, more numerous near antennae and ocelli; ocelli and ocularia black. Thorax and abdomen dirty yellowish-white, later becoming yellow-green. Thorax humped, twice as broad as middle segments of the tapering abdomen. Thoracic legs the same colour as the thorax, the coxae with a fine brown diagonal line. Prolegs on abdominal segments 2–7 and 10; well developed; unicolorous with body. Spiracles unicolorous with body.

Second Instar.—Average length 4.8 mm. (3.48 to 5.74 mm.). Average width of head capsule 0.52 mm. (0.47 to 0.55 mm.). Head orange, finely reticulated, with sparse, short, fine, light yellow setae; antennae light brown, slightly darker than capsule; mouthparts lighter in colour than capsule; ocelli and ocularia black. Thorax and abdomen yellow-green. Thorax humped and distinctly wider than the tapering abdomen; thoracic legs the same colour as the thorax, the coxae with a fine brown diagonal line, the tarsi brown. Abdomen with four indistinct and very fine longitudinal brown-green stripes; two just above the level of the spiracles and two laterad of the dorsal median line. Prolegs on abdominal segments 2–7 and 10; well developed; unicolorous with body. Spiracles yellow-green, slightly darker than body colour.

Third Instar.—Average length 6.38 mm. (5.65 to 7.83 mm.). Average width of head capsule 0.73 mm. (0.69 to 0.78 mm.). Head light orange-yellow, with sparse, fine, light yellow setae; antennae and mouthparts the same colour as the capsule; ocelli and ocularia black. Thorax and abdomen with dorsal aspect above spiracles light green and ventral aspect yellowish. Thorax humped and distinctly wider than the tapering abdomen. Thoracic legs yellowish, the coxae with a fine orange diagonal line, the tarsi dark brown. Four longitudinal brown-green stripes present, the two just above the spiracles being broader and extending

only the length of the abdomen, the two laterad of the dorsal median line being narrower and extending over the thorax and abdomen. Prolegs on abdominal segments 2-7 and 10; well developed: unicolorous with body. Spiracles unicolorous with the body.

Fourth Instar.—Average length 9.47 mm. (7.77 to 10.48 mm.). Average width of head capsule 1.08 mm. (1.02 to 1.13 mm.). Head orange, with sparse light yellow setae; antennae and mouthparts brown; ocelli and ocularia black.

Thorax and abdomen greyish-green on both dorsal and ventral aspects, the thorax humped and distinctly wider than the tapering abdomen. Four brown-green longitudinal stripes present, the two just above the spiracles being broader than the dorsal stripes on the abdomen and narrower than the dorsal stripes on the thorax, and discontinuous; the two laterad of the dorsal median line being continuous; all four stripes extending over the abdomen and thorax. Thoracic legs yellow-green, the coxae with a fine light brown diagonal line, the tarsi brown. Prolegs on abdominal segments 2-7 and 10; well developed; unicolorous with body. Spiracles lighter in colour than the body. Fine setae present on thoracic legs, prolegs and apex of anal segment.

Fifth Instar (Pl. I, fig. 3).—Average length 12.06 mm. (9.45 to 14.82 mm.). Average width of head capsule 1.32 mm. (1.26 to 1.36 mm.). Head orange with sparse, fine, yellow setae; frons surrounded by lighter-coloured area; antennae and labrum brown; ocelli and ocularia black. Thorax and abdomen green on both dorsal and ventral aspects, with scattered lighter-coloured setae each arising from a light green spot, the setae on the post-epipleurites being longer than the others. Four longitudinal brown-green stripes present, the two just above the spiracles being a darker shade than the two laterad of the dorsal median line and all four extending over the thorax and abdomen. The dorsal median line dark and transparent. The anterior and posterior margins of the segments each having a transverse, unpigmented, transparent line which imparts to the body a cross-banded appearance. Thoracic legs green, the coxae with a fine light brown diagonal line, the tibia yellow-brown, the tarsi brown. Prolegs on abdominal segments 2-7 and 10; well developed; unicolorous with body. Spiracles light brown. Apex of anal segment yellowish, glossy, with short setae.

Distribution and Forest Status.

A. destructor is widely distributed throughout many of the larch-growing areas of Britain and has been located in the following Forestry Commission forests:—Monaughty, Durriss, and Kirkhill in Aberdeenshire; Craig Vinean and Drummond Hill in Perthshire; Drumtochty in Kincardineshire; Glen Doll in Angus; Glentress, Cardrona, and Dreva in Peeblesshire; Wauchope and Newcastleton in Roxburghshire; Tinnisburn in Roxburghshire and Dumfriesshire; Forest of Ae and Auchenroddan in Dumfriesshire; Edgarhope in Berwickshire; Cairn Edward and Kirroughtree in Kircudbrightshire; Chopwell in Co. Durham; Warke, Rothbury, Kielder, Redesdale, Slaley, and Harwood in Northumberland; Kershope, Thornthwaite, and Greystoke in Cumberland; Grizedale in Lancashire; Mortimer in Herefordshire and Shropshire; Gynwyd and Dolgelly in Merioneth; Gwydyr in Caernarvon and Denbighshire; Radnor in Radnorshire; Myherin in Cardiganshire; Tarenig in Cardiganshire and Montgomery; Dovey in Merioneth and Montgomery; Hafren in Montgomery; Glasfynid and Brecon in Breconshire; Crychan in Breconshire and Carmarthenshire; Brecfa in Carmarthenshire; Dean in Gloucestershire; and Llanover in Monmouthshire. The species has also been taken on privately owned estates in Perthshire, Westmorland, Cumberland and Montgomery. Further studies will doubtless reveal that the distribution of the species is much wider than that given above.

All three larches commonly grown as forest crops in Britain, namely, European larch (*Larix decidua* Mill. (*europaea* D.C.), Japanese larch (*L. leptolepis* Murr.) and Hybrid larch (*L. eurolepis* Henry), are subject to attack by *A. destructor*. *L. occidentalis* Nuttall has also been found to be a food-plant for this species. Trees of between four and sixty-seven years of age have been attacked but infestation is usually most severe on crops of about 20 years of age. In general, trees situated on the perimeters and ride sides of plantations are most subject to attack and, on these, foliage damage progresses upwards during the growing season from the bottom of the crown where the heaviest egg-laying occurs. To date, no major epidemics of this species resulting in appreciable economic losses have occurred in Britain on any of the above mentioned tree species although infestations have frequently been severe enough to give rise to some degree of concern. One reason why the infestation of larch by *A. destructor* does not have serious results is that the larvae of this species, unlike those, for example, of *Pristiphora erichsoni*, do not completely devour the needles upon which they feed but instead more usually consume only portions of the individual needles. Consequently the attack does not result in the complete stripping of the tree crown or of parts of it but in a general reduction in density of the foliage, both on the long and short shoots. This type of feeding imparts to the crown of the attacked tree a distinct brown or "scorched" appearance but even this indication of damage does not appear until the level of the larval population is high. A technique for population estimation in use by the entomology section of the research branch of the Forestry Commission consists of jarring larvae from a sample branch in the lower part of the crown on to a sheet measuring 2 ft. 10 ins. by 1 ft. 10 ins. This "standard beat", as it is called, yields numbers of larvae per beat varying from zero to 100 or more. If these beats are taken before the larvae migrate, either to the upper parts of the crown for further feeding or to the soil for cocoon formation, it has been found that when the number of larvae collected from a standard beat is less than 30, foliage damage is not obvious and can be detected only by very close examination. When the number of larvae per standard beat falls between 30 and 80, browning of the foliage is obvious but not severe. It is only when the number of larvae per standard beat exceeds 80 that the browning of the foliage is severe. A second reason why attack by *A. destructor* seldom seems to result in a very high degree of damage to a tree is that the major portion of the feeding, which is carried out by the fourth- and fifth-instar larvae, takes place late in the growing season shortly before the tree normally loses its foliage.

Thus *A. destructor* does not appear at the present time to be a pest whose attacks will have any very serious repercussions upon the economic growing of larch in Britain. Its attacks must nevertheless result in some depression of the rate of growth of the host trees especially when, as is frequently the case, it occurs in association with other defoliators such as *Pristiphora laricis*, *P. erichsoni*, and *Coleophora laricella* (Hb.).

The only control which has been attempted has been along biological lines and concerns the release during 1950 of the Eulophid parasite, *Dahlbominus fuscipennis* (Zett.), in infested larch plantations in Perthshire. Stocks of this species, which parasitises a number of sawfly species, were originally obtained from Canada and after laboratory tests had shown that it was capable of successfully parasitising *A. destructor*, releases were effected in two ways. Firstly by direct release of the adult parasites in the plantation; and secondly, by placing in the plantations numbers of cocoons of *Neodiprion sertifer* (Geoffr.) which had been stocked with *D. fuscipennis* in the laboratories of the Forestry Commission Research Station. In these ways it is estimated that some 10,000 parasites were liberated in the plantations in question. No detailed study has been made of the effect of these liberations but it is certain that no spectacular degree of control was obtained since the infestations in these plantations in 1951 and 1952 continued on quite a severe scale.

Summary.

A new member of the larch-feeding genus *Anoplonyx* has recently been described in Britain. The species, *A. destructor* Benson, has been common in British larch woods for some years without, however, causing any very serious amount of damage to the tree crops. Notes on the biology of the species, including larval descriptions, are given and the present forest status of the species is reviewed. Apparently unsuccessful attempts to achieve biological control by the release of *Dahlbominus fuscipennis* (Zett.) are recorded.

Acknowledgements.

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The author has pleasure in acknowledging the assistance of the members of the staff of the entomology section of the research branch of the Forestry Commission ; and, in particular, that of Dr. K. Leius, whose drawing of the adult is reproduced in this paper and who carried out much of the laboratory work involved in the investigation.

References.

- BENSON, R. B. (1952). A new *Anoplonyx* destructive to larch in Britain.—Bull. ent. Res., **43**, pp. 543–547.
- FORESTRY COMMISSION (1950). Report on Forest Research for the year ending March, 1949, p. 23.
- FORESTRY COMMISSION (1951). *Ibid.*, 1950, pp. 87–88.



FIG. 1. *Anoplonyx destructor* Benson. Eggs on Larch (X 6 approx.).



FIG. 2. *A. destructor*, 1st instar larvae (X 6).



FIG. 3. *A. destructor*, lateral and dorsal view of full-grown larva (X 6 approx.).

LEAD CABLE SEVERELY DAMAGED BY *PTINUS TECTUS*
BOIELDIEU (COLEOPTERA, PTINIDAE).

By E. A. J. DUFFY, F.R.E.S. E.H.

Department of Entomology, British Museum (Natural History).

There are many published records of insects penetrating into or perforating various metals. In the majority of cases, however, the damage has been purely incidental, resulting from the blocking of the path of larvae or emerging adults by the metal, as when metal has encased wood that was infested prior to installation.

Recently the manager of a well-known cable factory in Kent sent to Mr. A. W. McKenny Hughes, of the British Museum, a sample of beetles and larvae which were said to be damaging one of their lead cables. Apparently, while running a cable of 1-in. diameter from one drum to another, it was noticed that the lead sheath, which was 0.06 in. thick, had been completely perforated in places and that thousands of small oval excavations up to one-eighth of an inch in diameter had been made in it,

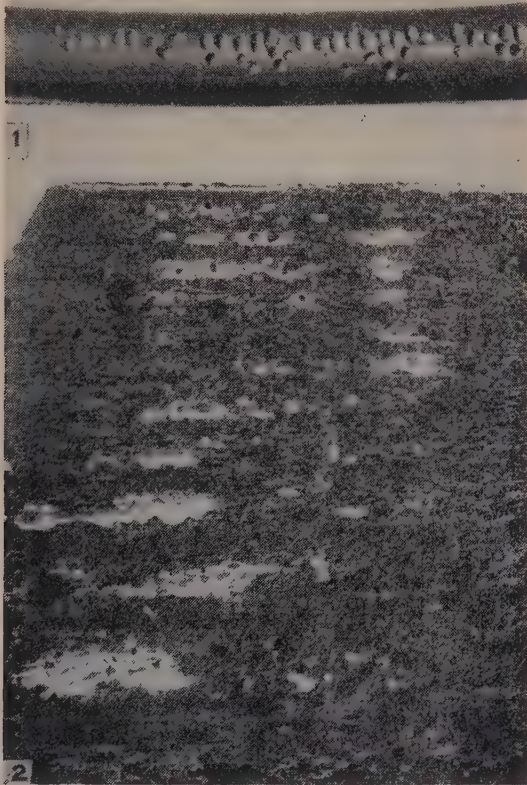


Fig. 1.—(1) Section of lead cable showing damage to the sheath caused by the construction of pupal cells by larvae of *Ptinus tectus* Boield. (2) Section of one of the cheeks of the cable drum showing damage caused by the construction of pupal cells by larvae of *P. tectus* Boield.

particularly where the lead had touched the hub of the drum or where consecutive turns of the sheath had been in contact. The sheath had been coated with bitumen and the cable stored under cover for about four years. On recognising the insects as *Ptinus tectus* Boield., both Mr. McKenny Hughes and I were far from convinced that this was in fact the species responsible for the damage attributed to it, and we therefore visited the factory.

There we were shown the cable, practically every foot of which bore well over a hundred pits, some of which were still occupied by larvae. Despite our doubts, the larvae proved to be *P. tectus*. The drum hub and the inner side of the cheeks were also heavily pitted and were almost honeycombed in places, and they were covered with a mixture of oil, sawdust and both dead and living beetles and larvae. Here, then, were hundreds of feet of cable completely ruined by an insect normally associated only with stored animal and vegetable products. The explanation was not at once apparent, but eventually the full facts came to light. The original wooden drum had been placed on its side for some months before the cable was re-wound, and it appeared that during this period some workmen had used the inside of the hollow hub as a receptacle for the remains of their lunches. The discarded food had served as an attraction to the beetles and subsequently as a breeding medium from which the mature larvae had escaped by entering the cavity half-way along the inside of the hub, through which the cable is anchored. Here, in order to pupate successfully, they had been forced to bore into the hub and the sheath of the cable to make their pupal cells. It appeared that in some cases this operation was successful but that the resulting adults, in their efforts to escape, had continued the boring process a stage further, completely perforating the sheath and causing the oil inside to seep through, and thus mummifying the remaining population.

Only two other species of the family PTINIDAE have been recorded damaging metals, namely, the cosmopolitan *Niptus hololeucus* (Fald.) and *Ptinus sexpunctatus* Panz. The former has been reported as injurious not only to lead but also to the quicksilver lining of the backs of mirrors and to silver plate and the gilt of chandeliers (Burke, Hartman & Snyder, 1922). *P. sexpunctatus* has been reported by Laing (1919) as being probably responsible for perforations in a leaden roof.

Summary.

An account is given of damage in Kent to a lead cable and its drum by larvae of *Ptinus tectus* Boield. The original infestation resulted from the accumulation of discarded food in the drum hub. The mature larvae, in order to pupate, bored into the hub of the drum and the sheath of the cable, thus producing innumerable excavations and some perforations in the sheath of several hundred feet of cable.

References.

- BURKE, H. E., HARTMAN, R. D. & SNYDER, T. E. (1922). The lead-cable borer or "short-circuit" beetle in California.—Bull. U.S. Dep. Agric., no. 1107, 56 pp.
- LAING, F. (1919). Insects damaging lead.—Ent. mon. Mag., **55**, pp. 278-279.
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A REVISED KEY TO THE LARVAE OF THE PTINIDAE ASSOCIATED WITH STORED PRODUCTS.

By D. W. HALL, B.Sc., F.R.E.S. E.H.

Zoology Department, St. Andrews University, University College, Dundee,*

and

R. W. HOWE, B.Sc., A.R.C.S., F.R.E.S.

Pest Infestation Laboratory, Department of Scientific & Industrial Research.

During the past 12 years, records of beetles of the family PTINIDAE as pests of stored products in Britain have been increasing in frequency. Kloet & Hincks (1945) record 21 British species of Ptinids, but of these, three species, *Ptinus palliatus* Perris, *P. pilosus* Mueller and *P. latefasciatus* Gorrh., have not so far been recorded from warehouses or stored products and a further two species, *P. lichenum* Marsh. and *P. subpilosus* Sturm have only rarely been recorded from such habitats. The importance of birds' nests as reservoirs of pests of stored products is being shown in a paper to be published shortly by Woodroffe and Southgate who carried out a survey of the fauna of the nests of birds associated with buildings. They found that the fauna of such a habitat was extraordinarily similar to that of warehouses in this country and that very large numbers of several species of Ptinids were present. The risk of this habitat being a possible source of many Ptinid infestations can be gleaned from the following list of species which they found :—

Ptinus tectus Boield., *P. fur* (L.), *P. sexpunctatus* Panz., *P. pusillus* Sturm, *P. subpilosus* Sturm, *Eurostus hilleri* (Reitt.), *Niptus hololeucus* (Fald.), *Mezium affine* Boield., *Trigonogenius globulus* Sol.

Manton (1945) produced a very satisfactory key to the larvae of 12 stored products species. For this, bred specimens were available in all species except *P. villiger* Reitt. which was described from larvae found associated with adults in a sack of flour. Since then we have bred and examined specimens of *P. villiger* and found that Manton's figures for this species agree with preparations made from mature larvae. *E. hilleri*, *M. affine* and *P. sexpunctatus*, have also been examined—species not available to Dr. Manton, and her key revised to incorporate these species. The larva of *P. sexpunctatus* was examined by Dr. H. E. Hinton, of Bristol University, and his modification of Manton's key to accommodate this species was included in a paper on its biology (Howe & Burges, 1951). A number of larvae of this species has been examined and in compiling the revised key two alterations have been made to that couplet.

The general characteristics of all the species considered in our revised key are compared in Table I, but it is especially interesting to note how *E. hilleri* and *M. affine* differ from certain closely related species.

The species *E. hilleri* and *N. hololeucus* resemble each other in having no empodial lobe on the claw of the tarsus and in the epipharynx having the main pair of diverging rows of setae with a large flat seta situated proximal to a long flat seta with a very blunt extremity. *E. hilleri* can be distinguished from *N. hololeucus* since it has only 3 sub-basal labral sensillae instead of 4, and also a long lip on the peritreme of the spiracles (figs. 27 & 28) which *N. hololeucus* has not.

*Now at Department of Scientific & Industrial Research, Pest Infestation Laboratory, Slough, Bucks.

The two species *M. affine* and *Gibbium psylloides* (Czenp.) are alike in having two strong setae on the prementum, but differ in that the former has the distal margin of the epipharynx slightly convex, whereas in the latter it is concave (figs. 52 & 53).

For completeness, figures are given for all the species included in Manton's key, with comparable figures for the three species mentioned above.

Manton's figures of the mandibles of *Ptinus latro* F., *P. raptor* Sturm, and *P. hirtellus* Sturm have been redrawn since these species were not available to us*. In the species examined it has been noted, almost without exception, the presence of a circular pit near the mesal margin. The presence of this and other morphological features in the mandible were not figured by Manton (1945). This character is absent in *P. villiger* and *P. pusillus* (figs. 3 & 5); the pit is situated some distance from the mesal tooth in *P. sexpunctatus*, *Tipnus unicolor* (Piller & Mitt.) and *Trigonogenius globulus* (figs. 7, 9 & 10) but in all the other species examined and figured (figs. 2, 8, 11-15) it is situated close to the mesal tooth. In three species in addition to this pit there is a groove or furrow also present. In *P. sexpunctatus* and *Tipnus unicolor* this groove is short and lies between the circular pit and the mesal tooth (figs. 7 & 9). In *P. pusillus* the groove is long and curved and situated near the posterior articulatory condyle (fig. 5).

Although, as pointed out by Manton (1945), there is a certain amount of individual variation in the shape of the preanal sclerite, we think it worth while to give a series of drawings of the general shape of this sclerite as certain shapes are characteristic for different species (figs. 81-92). Braune (1943) has shown in a schematic diagram "differences in the anal curve" of *P. tectus*, *P. fur*, *P. latro*, *P. sexpunctatus*, and *P. hirtellus*, and has attempted to draw up a key to the larvae of these species on the shape and coloration of this character. There is, however, considerable intra-specific variation in the coloration of the preanal sclerite and this coloration can vary even during the life of a larva, e.g., as Braune states himself it is always more strongly coloured just prior to moulting and metamorphosis. Our figures of the shapes of the preanal sclerites of the above species are not in complete agreement with those given by Braune. The presence of a preanal sclerite is not the most reliable identification mark for Ptinid larvae as this is also characteristic of some of the Anobiids, such as *Lasioderma serricorne* (F.). A key by which Ptinid larvae can be distinguished from other families of the Bostrychoidea has been given by Manton (1945) and shows that the head is not retracted into the prothorax, the thoracic spiracle is in the antero-lateral part of the prothorax, and the dorsal surface of the abdomen does not have bands of spicules or stout short setae.

Table I lists some of the larval characters in such a way that those most easily examined are considered first, and it is thought that by using this table the identification of a Ptinid larva could be quickly made although probably not so accurately as with the complete key given in this paper.

KEY TO MATURE OR NEARLY MATURE LARVAE OF THE PTINIDAE.

1. Preanal sclerite large, U-shaped, reaching to about the middle of the anal groove (fig. 81).....2
- Preanal sclerite small, variable in size and shape, the arms of the triangular or slightly U-shaped sclerite too short to embrace more than the extreme end of the anal groove (figs. 84-92).....7
2. Labrum with 4 sub-basal sensillae (fig. 46)*Ptinus hirtellus* Sturm
- Labrum with 3 sub-basal sensillae (figs. 41-45).....3

*We have now examined *P. hirtellus* and find it has a pit.

Summary of the characters

Character	Ptinus latro	Ptinus fur	Ptinus villiger	Ptinus raptor	Ptinus pusillus	Ptinus hirtellus
A. <i>Preanal Sclerite</i> .						
1. Shape	U narrow (Braune—1948)	U	U	U	U	U arms unequal
B. <i>Leg</i> .						
2. Empodial lobe (present+ ; absent—)	+	+	+	+	+	+
3. Tibia—number of setae	12–13	8–13	7–8	21–23	19–20	12–15
4. Tibia—dorsal proximal area free of setae	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{3}$	0	0
C. <i>Spiracle—1st abdominal</i> .						
5. Maximum length excluding lip in mm.	0·02	0·02	0·07	?	0·03	0·02
6. Maximum width /width of lip ...	2·5–3·0	2·5	6	2·5–3·0	3·5	2·5–3·0
D. <i>Mandible</i> .						
7. Mesal tooth—angle	acute	acute	acute	acute	acute	acute
8. Maximum length /maximum width ...	1·16–1·20	1·16–1·20	1·20–1·23	1·11	1·16–1·20	1·20
9. Maximum length /spiracle width ...	14	11	12	11	11	14
E. <i>Labium</i> .						
10. Pairs of setae on postmentum ...	1	1	1	1	1	1
11. Pairs of setae on prementum ...	8	6	7–8	7–9	7–8	5–6
12. Pairs of setae projecting between palps	1	2	2	1–2	2	2
F. <i>Epipharynx</i> .						
13. Number of labral sensillae	3	3	3	3	3	4
14. Inner arm of Y-shaped sclerite ...	long pointed	long narrow	short knob-like	long pointed	Intermediate narrow 3–8	long narrow
15. Number of setae between Y sclerites	1	1–2	3	5	3–8	3
16. Number of setae in diverging row ...	7	6–8	5	6–9	6–9	5–7
17. Number of setae in lateral row ...	0	0	0	0	0–6	0

LE 1.

of the larvae of the Ptinidae.

Ptinus expunctatus	Ptinus tectus	Tipnus unicolor	Trigonogenius globulus	Stethomezium squamosum	Mezium affine	Gibbium psylloides	Eurostus hilleri	Niptus hololeucus
small V	small	small	small	small	small V	small	small	small
+	+	+	+	+	+	+	—	—
14	8-13	8-13	11-23	5-7	11	8-13	13	10-15
$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{3}$	0	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0
0.04	0.03	0.025	0.02	0.006	0.02	0.03	0.02	0.06
2.5-3.5	3	2-3	1.5	1.5-2.0	2.0-2.5	3	2.0-2.5	4.5
acute	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse	obtuse
1.35	1.2	1.2	1.2	1.33-1.35	1.25	1.25	1.2	1.35
9	13	10	18	40	18	15	16	18
1	1	1	1	1	absent	absent	1	1
5-6	2	1-2	5-6	1	11-14+1 strong	3+1 strong	6	8-9
3	4	1	2	1	2	1	1	2
3	3	3	3	3	3	3	3	4
long medium	long broad	Intermediate medium	Short medium	Variable	Short broad	Short broad	Long broad pointed	Intermediate broad
4-5	0	1	2-7	0	0	0	0-5	7
7-8	5	5	7-9	4-5	3-4	5-6	4	4
0	7	0	11	0	2-4	2-3	5-6	12-15

3. Width of the first abdominal spiracle (fig. 18) about $6\times$ that of its lip ; spiracles large, length of mandible being about $6\times$ the longest diameter of the first abdominal spiracle. The inner arm of the Y-shaped sclerite in the labrum very much shorter than the outer arm, knob-shaped (fig. 43). Tibia with setae absent from the proximal half of the upper surface, and bearing about 7-8 setae (fig. 72).....*Ptinus villiger* Reitt.
- Width of the first abdominal spiracle (figs. 16, 17 & 20) about $2-3\frac{1}{2}\times$ that of its lip ; spiracles medium sized, length of mandible being $8-14\times$ the longest diameter of the first abdominal spiracle. The inner arm of the Y-shaped sclerite in the labrum (figs. 41, 42, 44 & 45) as long or almost as long as the outer arm. Tibia with setae present on the proximal half of the upper surface (figs. 70, 71 & 73).....4
4. Tibia with setae absent from the proximal quarter of the upper surface (figs. 71 & 73).....5
Tibia with setae present on the proximal quarter of the upper surface (fig. 70)....6
5. Tibia with setae extending dorsally along the whole of the distal two-thirds or three-quarters, total number about 21-23 (fig. 73). Tooth on the mesal margin of the mandible (fig. 4) slightly smaller and projecting markedly less than in *P. fur*.....*Ptinus raptor* Sturm
- Tibia with setae forming a band round the middle which is separated by a gap from two dorsal setae near the distal end, total number 8-13 (fig. 71). Tooth on the mesal margin of the mandible (fig. 2) slightly larger than in *P. raptor* and projecting acutely.....*Ptinus fur* (L.)
6. Tibia with about 19-20 setae. The epipharynx (fig. 45) with the main pair of diverging rows of setae numbering about 6-9 and sharper than in *P. latro* ; with the setae lateral to the diverging rows numbering 0-6 and tending to form one subsidiary row parallel to the diverging rows ; with a basal median group of 3-8 small setae ; and with the setae composing the irregular transverse rows near the front margin, between the diverging rows, tapering and sharp and more numerous than in *P. latro*.....*Ptinus pusillus* Sturm
- Tibia with about 12-13 setae. The epipharynx (fig. 41) with the main pair of diverging rows of setae numbering about 7 and blunter than in *P. pusillus* ; with setae lateral to the diverging rows usually absent ; with the basal median group usually represented by one seta ; and with the setae composing the irregular transverse rows near the front margin, between the diverging rows, blunter and fewer than in *P. pusillus*.....*Ptinus latro* F.
7. Claw of tarsus without empodial lobe (figs. 79, 80). Epipharynx with main pair of diverging rows of setae each bearing a large flat seta situated proximal to a long flat seta with a very blunt extremity (figs. 54 and 55).....8
Claw of tarsus with empodial lobe (figs. 70-78). Epipharynx not as above.....9
8. Labrum with 4 sub-basal sensillae (fig. 55). Epipharynx with main pair of diverging rows of setae each bearing 4 strong setae ; 2nd pair of setae are large, flat and pointed ; 3rd pair of setae are long, flat, and with a blunt extremity (fig. 55). Spiracles (fig. 28) with no lip (sometimes short lip) on the peritreme of 1st abdominal segment ; width of the first abdominal spiracle is about $4\frac{1}{2}\times$ that of its lip when present. Upper side of labium (fig. 69) with a transverse row of 3-5 setae at the base of the palp.....
Niptus hololeucus (Fald.)
- Labrum with 3 sub-basal sensillae (fig. 54). Epipharynx with main pair of diverging rows of setae each bearing 4 strong setae ; 1st and 2nd pairs of

- setae are large, flat and rounded at the extremity : 3rd pair of setae are long, flat and with a blunt extremity (fig. 54). Spiracles with a long lip on the peritreme ; width of the first abdominal spiracle is about $2\frac{1}{2} \times$ that of its lip (fig. 27). Upper side of labium (fig. 68) without a transverse row of setae at the base of the palp.....*Eurostus hilleri* (Reitt.)
9. Inner arm of Y-shaped sclerites in the labrum (figs. 50 & 52) considerably shorter than outer arm10
Arms of Y-shaped sclerites in the labrum (figs. 48 & 53) approximately equal in length11
10. Claw of tarsus (fig. 76) bearing a few (1-3) setae on the posterior side in addition to the seta on the antero-mesal border. Epipharynx with about 11 basal median setae, and about 10 setae lateral to each diverging row. The lip on the peritreme of the spiracles (fig. 23) on most segments is wider towards the base and the sides tend to converge instead of being parallel on many segments.....*Trigonogenius globulus* Sol.
Claw of tarsus (fig. 78) bearing no setae on the posterior side in addition to the seta on the mesal or antero-mesal border. Upper surface of the labium (fig. 66) with one pair of strong setae near the middle line, at least twice as wide as other setae on the prementum. Epipharynx with no basal median setae and less than 7 setae lateral to each diverging row. Lip on the peritreme of the spiracles approximately parallel sided and not markedly wider near its base (fig. 25).....*Mezium affine* Boield.
11. Distal margin of the epipharynx (fig. 53) distinctly concave in the middle line. The upper surface of the labium (fig. 67) with one pair of strong setae near the middle line, at least twice as wide as other setae on the prementum, and about 1-2 additional setae ; with about 3 setae on each side across the base of the prementum ; and with a few setules on the basal segment of the palp and prementum.....*Gibbium psylloides* Czenp.
Distal margin of the epipharynx (figs. 47 & 48) convex or straight in the middle line. Labium not as above.....12
12. Epipharynx (fig. 47) with setae lateral to the diverging rows absent. The upper side of the labium (fig. 61) bearing no strong setae near and in addition to the pair near the middle line.....13
Epipharynx (fig. 48) with setae lateral to the diverging rows present ; no median group of setae present between the inner arms of the Y-shaped sclerites. The upper side of the labium (fig. 62) bearing several strong setae near and in addition to the pair near the middle line ; about 4 pairs of setae project beyond the anterior margin of the prementum between the palps. Mandible (fig. 8) wide, the length being about $1.2 \times$ the width ; tooth on the mesal margin obtuse.....*Ptinus tectus* Boield.
13. Epipharynx (figs. 49 & 51) small, less than 130μ wide at the transverse fold. Tooth on the mesal margin of the mandible obtuse. The upper side of the labium with 1-2 setae on each side across the base of the prementum ; and with about 1 pair of setae projecting beyond the anterior margin of the prementum between the palps.....14
Epipharynx larger, about 160μ wide at the transverse fold. Tooth on the mesal margin of the mandible acute. The upper side of the labium with 4-5 setae on each side across the base of the prementum ; and with 3 pairs of setae projecting beyond the anterior margin of the prementum between the palps (fig. 61).....*Ptinus sexpunctatus* Panz.

14. Claw of tarsus (fig. 75) with a small empodial lobe which may be ill-defined ; the base of the brown part of the claw not markedly expanded on the mesal side. Setae on the tibia absent from the proximal quarter of the dorsal surface, and about 8-13 in number. The two proximal pairs of setae on the diverging rows on the epipharynx (fig. 49) almost the same distance apart. Mandible (fig. 9) wide, the length being about $1.2 \times$ the width ; the tooth on the mesal margin obtuse, its point being as near to the base as to the apex of the mandible. Tactile appendix of antenna (fig. 34) almost twice as long as wide. The upper side of the labium (fig. 63) bearing one pair of strong setae near the middle line, at least twice as wide as other setae on the prementum ; and with other setae on the prementum ; and with a few setules on the prementum and basal segment of the palp. Spiracles medium sized, the length of the mandible being about $10 \times$ the diameter of the first abdominal spiracle..... *Tipnus unicolor* (Piller & Mitt.)

Claw of tarsus (fig. 77) with a large clearly defined empodial lobe ; the base of the brown part of the claw markedly expanded on the mesal side distal to the seta. Tibia with setae present on the proximal quarter of the dorsal surface and numbering about 5-7. The basal pair of setae of the diverging rows on the epipharynx (fig. 51) separated by a distance of half that which separates the next pair of setae. Mandible (fig. 11) narrower than in *T. unicolor*, length being about $1\frac{1}{2} \times$ the width ; tooth on the mesal margin obtuse, its point being nearer to the apex than to the base of the mandible ; the apex of the mandible more curved than in *T. unicolor*. Tactile appendix of the antenna slightly longer than wide (fig. 36). The upper side of the labium (fig. 65) bearing one pair of setae near the middle line which are not stronger than other setae on the prementum ; no setules present on the prementum or on the basal segment of the palp. Spiracles (fig. 24) small, the length of the mandible being about $40 \times$ the diameter of the first abdominal spiracle..... *Stethomezium squamosum* Hinton

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We are greatly indebted to Dr. S. M. Manton for allowing us to add to her published key. Some of the present figures are based on the figures that accompanied her original key.

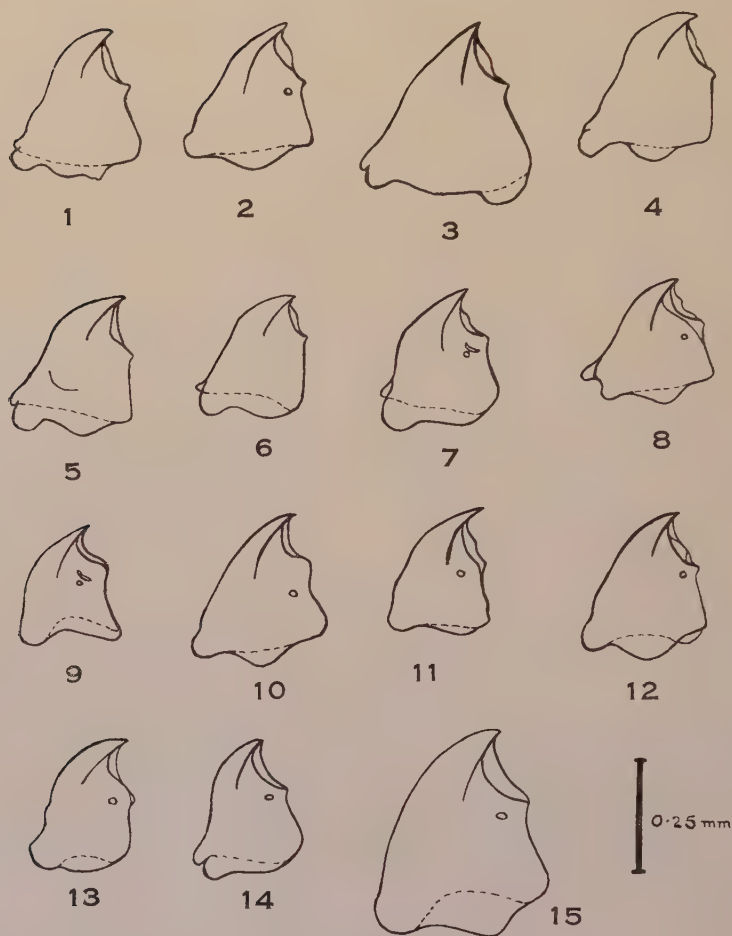
We are most grateful for the active interest taken in the work by Professor A. D. Peacock in whose Department at St. Andrews University, University College, Dundee, some of the work has been done, and to the Bowden Entomological Research Fund, University of Glasgow, for financial assistance.

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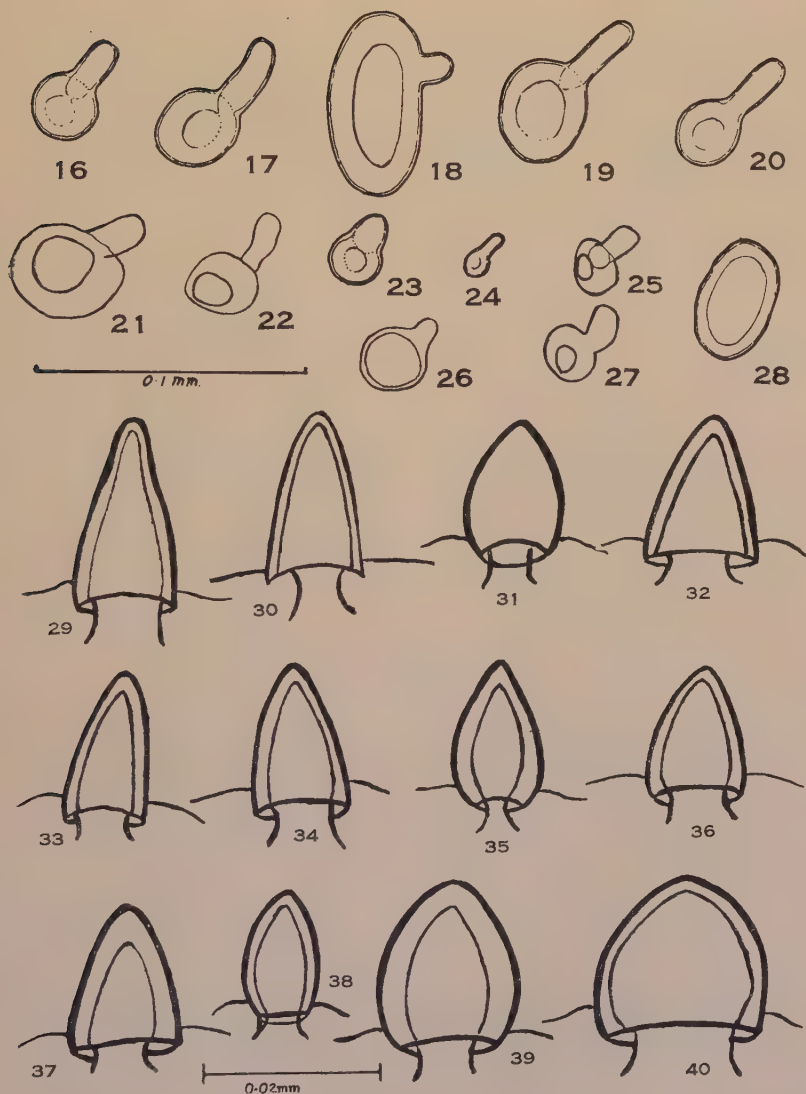
References.

- BRAUNE, R. (1943). Vergleichende Untersuchungen an den Diebskäfern *Ptinus tectus* Boield., *Ptinus fur* L., *Ptinus latro* Fabr., *Ptinus sexpunctatus* Panz. und *Ptinus brunneus* Duft., zugleich der experimentelle Beweis für die Notwendigkeit des Flüssigkeitsausgleichs im Insektenkörper.—Z. Morph. Oekol. Tiere, **39**, pp. 546-691.
- HOWE, R. W. & BURGESS, H. D. (1951). Studies on beetles of the family Ptinidae, VI. The biology of *Ptinus fur* (L.) and *Ptinus sexpunctatus* Panzer.—Bull. ent. Res., **42**, pp. 499-511.

KLOET, G. S. & HINCKS, W. D. (1945). A check list of British insects. Stockport.
 MANTON, S. M. (1945). The larvae of the Ptinidae associated with stored products.
 —Bull. ent. Res., **35**, pp. 341–365.

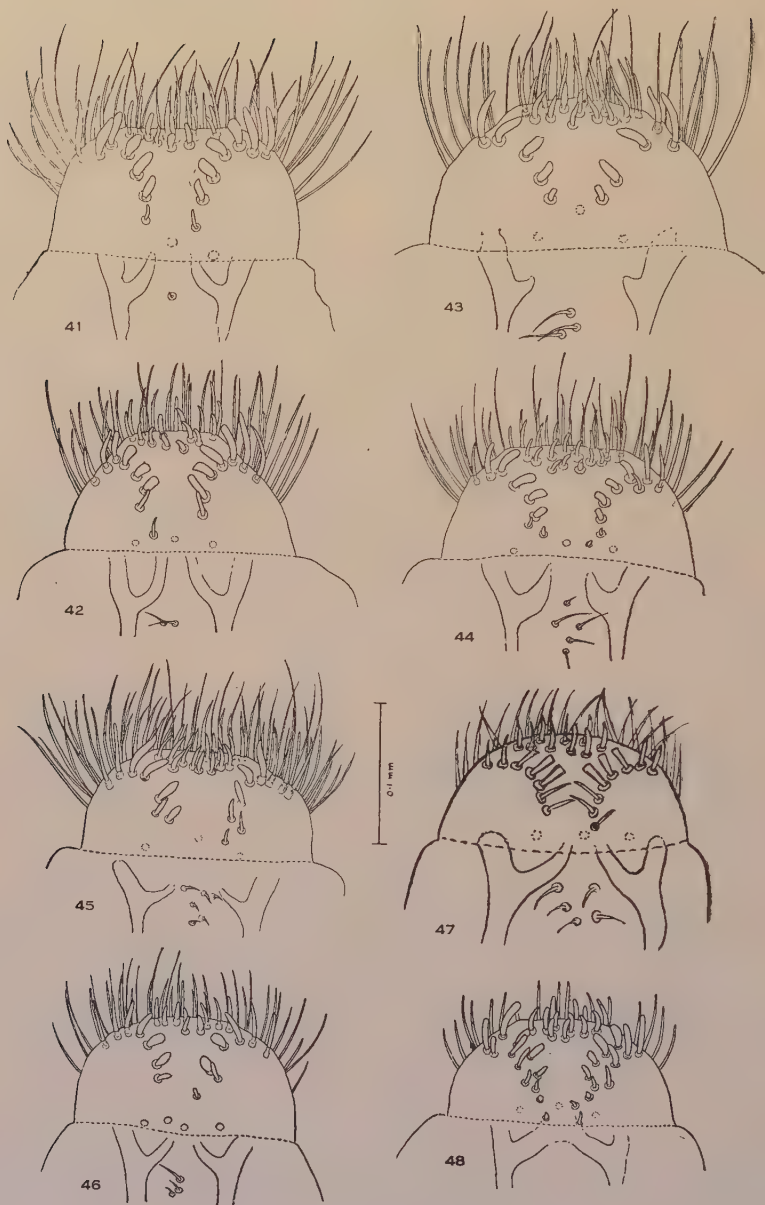


Figs. 1–15.—Mandibles : (1) *Ptinus latro* ; (2) *P. fur* ; (3) *P. villiger* ; (4) *P. raptor* ; (5) *P. pusillus* ; (6) *P. hirtellus* ; (7) *P. sexpunctatus* ; (8) *P. tectus* ; (9) *Tipnus unicolor* ; (10) *Trigonogenius globulus* ; (11) *Stethomezium squamosum* ; (12) *Mezium affine* ; (13) *Gibbium psylloides* ; (14) *Eurostus hilleri* ; (15) *Niptus hololeucus*.

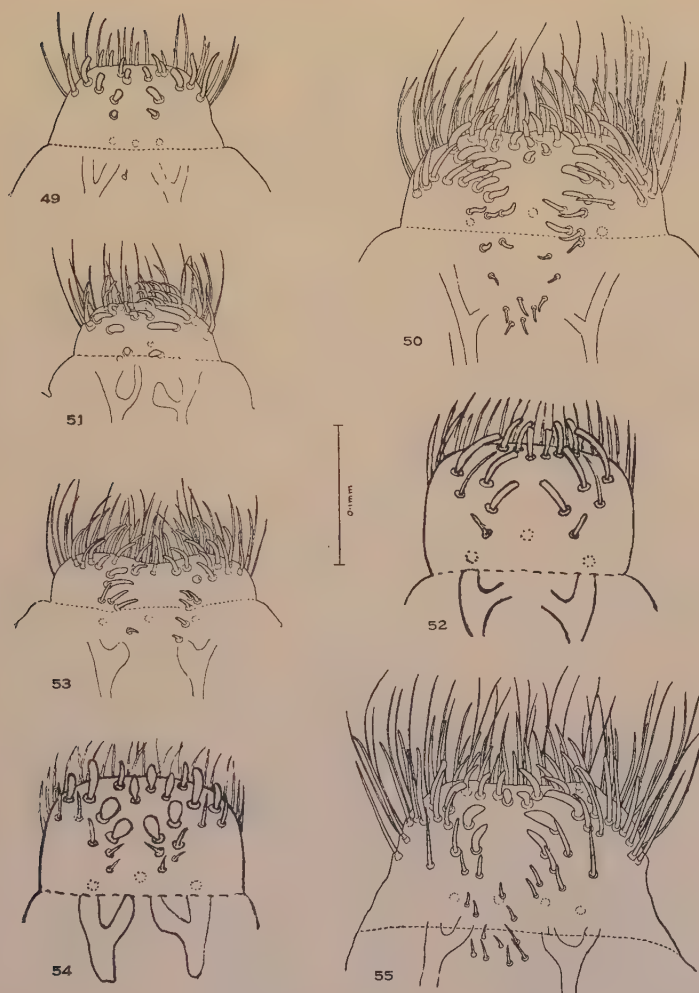


Figs. 1-28.—Peritreme of first abdominal spiracle: (16) *Ptinus latro*; (17) *P. fur*; (18) *P. villiger*; (19) *P. pusillus*; (20) *P. hirtellus*; (21) *P. sexpunctatus*; (22) *P. tectus*; (23) *Trigonogenius globulus*; (24) *Stethomezium squamosum*; (25) *Mezium affine*; (26) *Gibbium psylloides*; (27) *Eurostus hilleri*; (28) *Niptus hololeucus*.

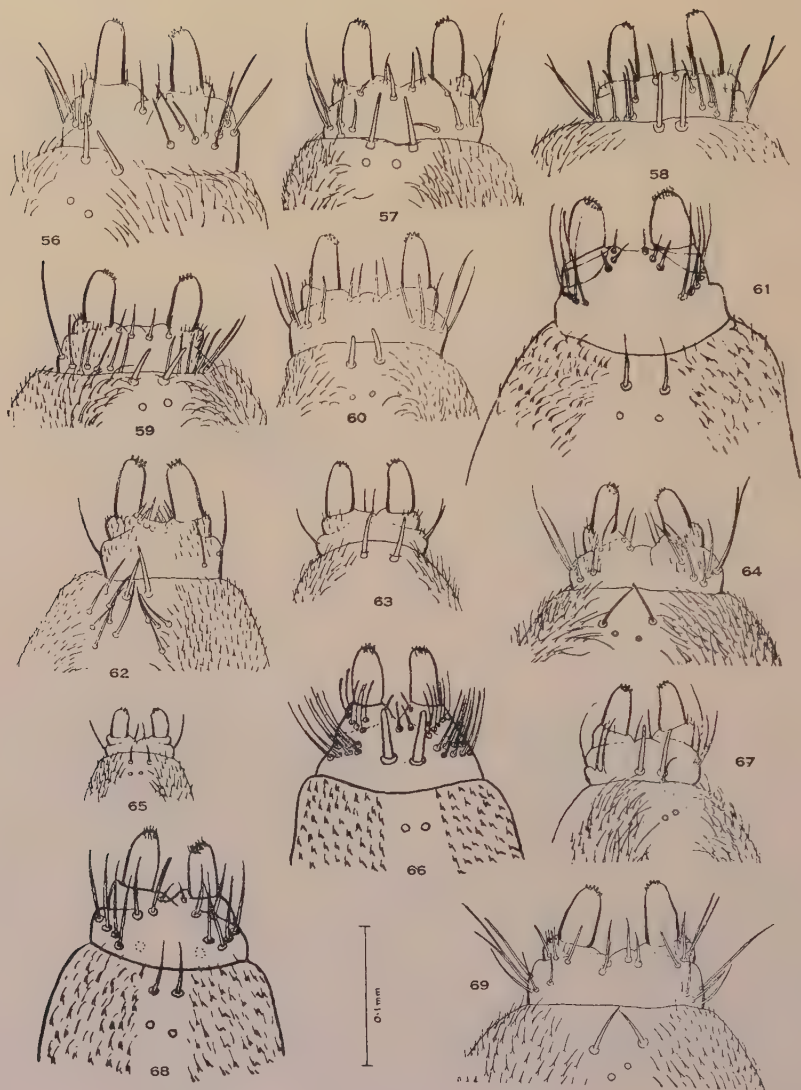
Figs. 29-40.—Tactile appendix of antenna: (29) *Ptinus fur*; (30) *P. villiger*; (31) *P. pusillus*; (32) *P. sexpunctatus*; (33) *P. tectus*; (34) *Tipnus unicolor*; (35) *Trigonogenius globulus*; (36) *Stethomezium squamosum*; (37) *Mezium affine*; (38) *Gibbium psylloides*; (39) *Eurostus hilleri*; (40) *Niptus hololeucus*.



Figs. 41-48.—Epipharynx, with labral sensillae seen through the epipharynx shown by dotted circles. The distribution of the setae on the dorsal surface only is given. (41) *Ptinus latro*; (42) *P. fur*; (43) *P. villiger*; (44) *P. raptor*; (45) *P. pusillus*; (46) *P. hirtellus*; (47) *P. sexpunctatus*; (48) *P. tectus*.



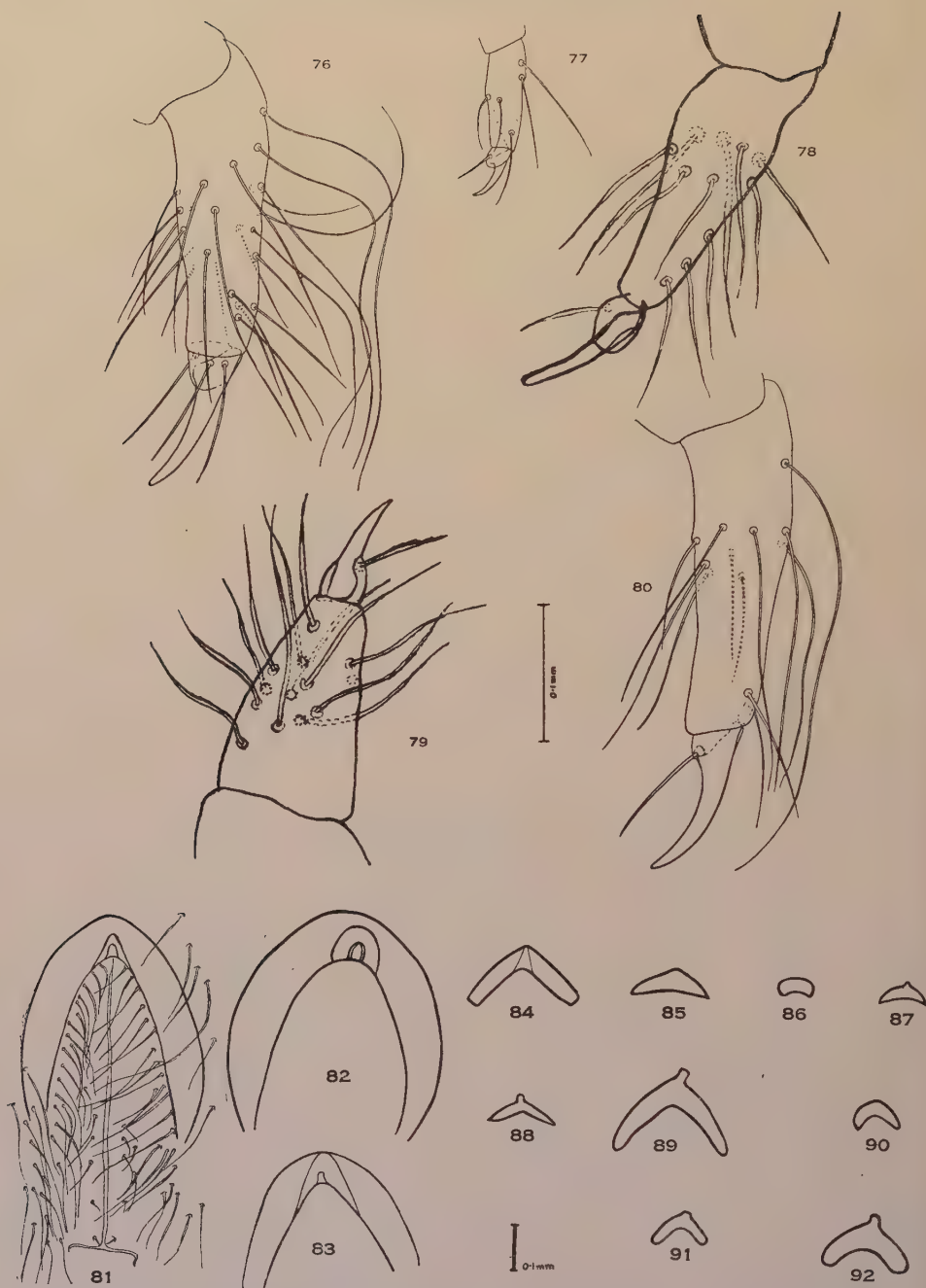
Figs. 49–55.—Epipharynx, with labral sensillae seen through the epipharynx shown by dotted circles. The distribution of the setae on the dorsal surface only is given. (49) *Tipnus unicolor*; (50) *Trigonogenius globulus*; (51) *Stethomezium squamosum*; (52) *Mezium affine*; (53) *Gibbium psylloides*; (54) *Eurostus hilleri*; (55) *Niptus hololeucus*.



Figs. 56-69.—The upper side of labium showing setae on that surface only. (56) *Ptinus latro*; (57) *P. fur*; (58) *P. raptor*; (59) *P. pusillus*; (60) *P. hirtellus*; (61) *P. sexpunctatus*; (62) *P. tectus*. (63) *Tipnus unicolor*; (64) *Trigonogenius globulus*; (65) *Stethomezium squamosum*; (66) *Mezium affine*; (67) *Gibbium psylloides*; (68) *Eurostus hilleri*; (69) *Niptus hololeucus*.



Figs. 70-75.—First leg, posterior view, setae on the anterior side seen through the limb shown by dotted lines. (70) *Plinus latro*; (71) *P. fur* (*P. tectus* and *Gibbium psylloides* are also like this); (72) *P. villiger*; (73) *P. raptor*; (74) *P. sexpunctatus*; (75) *Tipnus unicolor*.



Figs. 76-80.—First leg, posterior view, setae on the anterior side seen through the limb shown by dotted lines. (76) *Trigonogenius globulus*; (77) *Stethomezium squamosum*; (78) *Meziium affine*; (79) *Eurostus hilleri*; (80) *Niptus hololeucus*.
 Figs. 81-92.—Schematic drawings of preanal sclerite. (81) *Ptinus fur*, showing relationship of sclerite to the preanal groove and the associated chaetotaxy; (82) *P. fur*; (83) *P. villiger*; (84) *P. sexpunctatus*; (85) *P. tectus*; (86) *Tipnus unicolor*; (87) *Trigonogenius globulus*; (88) *Stethomezium squamosum*; (89) *Meziium affine*; (90) *Gibbium psylloides*; (91) *Eurostus hilleri*; (92) *Niptus hololeucus*.

TRUNK ABSORPTION OF A SYSTEMIC CHEMICAL BY COFFEE.

By J. A. B. BOND.

(PLATE II.)

An investigation in Kenya in 1951-52 into the control of the coffee mealybug, *Planococcus kenyae* (Le Pelley), was carried out on behalf of the Coffee Board of Kenya in various coffee plantations and included methods of chemical control by a systemic insecticide. In the first place, the methods used by Hanna on cacao in the Gold Coast were tried (West, 1951 ; Hanna & others, 1952). For this purpose, the root absorption of Hanane (which consists mainly of bisdimethylamino fluoro-phosphine oxide) was investigated by applying the chemical to the soil in a groove around the base of the tree. Contrary to Hanna's results on cacao in the tropical rain forests of the Gold Coast, the results on coffee proved less consistent.

Experiments were therefore initiated in February and March 1952 to investigate other possible methods of application including trunk injection and trunk absorption of Hanane and other systemic insecticides ; these methods were more effective and consistent than soil treatment.

Jeppson & others (1952) and Metcalf and March (in press), working recently on citrus trees and seedlings, have shown that Systox, of which the principal active ingredient is O-(2-(ethylmercapto)ethyl) O,O-diethyl thiophosphate, and schradan (octamethylpyro-phosphoramide) applied to the bark are readily absorbed and translocated, and that the efficiency of absorption of schradan is somewhat greater than that from applications in water culture.

The technique developed in trunk absorption tests on mature coffee trees, *Coffea arabica*, was as follows (Pl. II). A section of the trunk about 4 ins. wide and a few inches above soil level was scraped with a knife or stiff brush to remove all dirt and loose bark. A 6-in. wide band of oil silk was placed around the base of the trunk with plenty of overlap at the join and the top edge of the silk tied tightly round the lower edge of the scraped area. A double thickness of surgical lint was wrapped around the scraped area to form a complete band, the bottom of which fitted into the fold in the oil silk formed when its lower edge was pulled up to make a waterproof cover over the lint. Any liquid not absorbed immediately by the lint collected in the fold and was thus prevented from running down the trunk into the soil.

In the first treatment using this technique a very high dosage of Hanane was applied, *viz.*, 250 cc. containing 32.5 grammes of active ingredient. The tree was inspected daily ; five days after treatment leaf edge necrosis, characteristic of the phytotoxic effects of Hanane, began to appear on most of the branches and at seven days damage was considerable. After 14 days the tree was almost completely defoliated, only the terminal shoot on one of the three primary stems remaining green. It is interesting to note here that in several highly replicated experiments carried out in 1951 the same and higher dosages of Hanane applied to the soil for root absorption had caused little or no phytotoxic damage in most cases.

Two more trees, well infested with mealybug, were treated with 2 and 5 grammes of active ingredient respectively in 200 cc. ; no phytotoxic effects were noticeable but a drop in the degree of infestation on both trees was in evidence after two weeks, and no mealybugs could be found after 12 weeks, although control trees remained well infested during the same period. Results are given in Table I. An index

system was used to assess the degree of infestation with *P. kenyae* on each tree before and after treatment as follows :—

Index No.	Degree of Infestation.
0	No mealybugs on tree.
1	Single mealybugs only, no colonies.
2	Less than 20 colonies.
3	More than 20 colonies but only parts of tree infested.
4	Overall infestation of tree.

TABLE I.
Summary of Hanane treatments by trunk absorption method.

Gm. active ingredient applied	Infestation indices before and after treatment							Notes
	Pre- treatment	Weeks after treatment						
		1	2	3	4	8	12	
(controls) 0	4	4	4	4	3.7	3.3	2.7	Average of 3 trees
2	4	4	3	2	2	1	0	Single tree
5	4	4	3	2	2	1	0	Single tree
8	4	1	0	0	0	0	0	Average of 2 trees
10	4	2	1	1	1	0	0	Average of 2 trees

Dosages were increased in further experiments to find the level at which phytotoxicity commenced ; with 6, 8 and 10 grammes of active ingredient absorption and translocation to the foliage were proved by good kills of mealybug. Little or no damage occurred on the trees, although yellowing of the foliage was noticeable on most of them.

Another test was conducted to find out whether removal of the bark before treatment would affect the rate of absorption and translocation. Two trees were each treated with 8 grammes of active ingredient in 200 cc. The trunk of one tree was completely ring barked for a width of 4 ins. before applying the band so that the chemical would be in direct contact with the cambium layer ; the bark of the other tree was only lightly scraped as in previous treatments. No live mealybugs could be found on either tree two weeks after treatment, but widespread damage to the foliage was noticeable after six days on the ring-barked tree and almost complete defoliation after three weeks, whereas no phytotoxic effects were observed on the other tree.

In March 1952, an experiment was carried out with the object of comparing directly trunk and soil treatments under the same conditions. Hanane was applied by both methods using two dosages, 3 and 6 grammes of active ingredient in 200 cc. per tree, with 3 replicates in each treatment. The soil treatment consisted of pouring the diluted insecticide into a shallow groove around the base of the trunk, a metal cone being used to prevent a rapid dispersal of the solution laterally. The trunk applications were made as described previously but with very little abrasion of the bark before treatment.

No phytotoxic damage was noticeable with any of the treatments. Significant changes in the mealybug populations did not appear at the lower dosages but marked differences were shown in the case of the high dosage treatments and are given in Table II ; at three weeks after treatment a drop in mealybug infestation was recorded for two of the three trunk-treated trees, whereas no change had occurred in the case of the soil-treated trees. Two of the latter were therefore given a second soil application at the same dosage. At six weeks all the trunk-treated trees showed reduction

of infestation to a very low level, one tree being completely clear. The two trees re-treated by the soil method also showed a reduction, but the third tree which had received only one application by this method again showed no change. A slight increase in infestation was seen on the control trees during the same period.

TABLE II.

Comparative effects of trunk and root absorption of Hanane by coffee trees on infestations of P. kenyae.

Method of application	Gm. active ingredient	Infestation indices before and after treatment							Notes
		Pre-treat-ment	Weeks after treatment						
			1	2	3	4	6	8	
To soil ...	6	3	3	3	3	3	3	3	Single tree
To soil (double)	12	3.5	3.5	3.5	3.5	2.5	2	1	Average of 2 trees
To trunk ...	6	4	4	3.5	3	2	1	0.3	Average of 3 trees
Controls ...	0	3.7	3.7	3.7	4	4	4	3	Average of 3 trees

The second application of 6 grammes active ingredient to the soil was made after the counts at the end of the third week.

Application of the chemical to the trunk without the waterproof and absorbent bands was found to be unsatisfactory, no doubt owing to the high volatility of the material used. The best absorption and translocation occurred when the treated area of the trunk was well scraped before application, exposing the cambium layer in several places.

Summary.

The systemic insecticide Hanane (mainly bisdimethylamino fluorophosphine oxide) is readily absorbed by the trunk of a coffee tree and translocated to the foliage when the bark is lightly scraped and the chemical applied in solution on a pad of absorbent material covered with a waterproof skin to prevent evaporation.

Smaller dosages of Hanane are required to give equivalent control of *Planococcus kenyae* (Le Pelley), or to cause a similar degree of phytotoxic damage, by this method than by soil treatment.

Abrasion or removal of the bark before application enables a greater absorption of the chemical but leads to serious phytotoxic damage by a comparatively low dosage.

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References.

- HANNA, A. D., HEATHERINGTON, A. & JUDENKO, E. (1952). Control of the mealy-bug vectors of the swollen shoot virus by a systemic insecticide.—*Nature*, Lond., **169**, pp. 334-335.
- JEPSON, L. R., JESSER, M. J. & COMPLIN, J. O. (1952). Tree trunk application as a possible method of using systemic insecticides on citrus.—*J. econ. Ent.*, **45**, pp. 669-671.
- WEST, J. (1951). Progress of work at WACRI on systemic insecticides carried out with Pest Control, Ltd.—*Rep. Cocoa Conf.*, 1951, pp. 86-92.



TREATMENT OF THE TRUNK OF A COFFEE TREE WITH HANANE.

FIELD OBSERVATIONS ON THE CACAO MIRIDS, *SAHLBERGELLA SINGULARIS* HAGL. AND *DISTANTIELLA THEOBROMA* (DIST.), IN THE GOLD COAST.

PART I. MIRID DAMAGE.

By G. WILLIAMS, B.Sc.

*West African Cacao Research Institute, Tafo.**

The two major obstacles to the growing of cacao (*Theobroma cacao* L.) in the Gold Coast are the virus complex known as swollen shoot and the cacao Mirids. Control of these was accepted as the immediate programme of research for the West African Cacao Research Institute from its inception (Voelcker, 1948), and numerous papers on swollen shoot and the mealybug vectors of it have already appeared. With the exception of a general paper by Squire (1947), information about the Mirid pests is confined to the Annual Reports of the West African Cacao Research Institute and other Government publications, and these have only a limited circulation.

Rational control methods must be based upon knowledge of the habits of the insects involved, and the papers in this series summarise such information. The writer was engaged on this investigation from 1947 to 1950, but some of the observations began as early as 1944 and these earlier records have been freely used where necessary.

A complete account of the West African cacao Mirids would include four species, all members of a single sub-family, the Bryocorinae, *Sahlbergella singularis* Hagl., *Distantiella theobroma* (Dist.), *Bryocoropsis laticollis* Schum. and *Helopeltis bergrothi* Reut. Only the first two species are of economic importance, however, and the other two can therefore be considered more summarily. Both *B. laticollis* and *H. bergrothi*, when present on cacao, are virtually confined to the developing green pods, though both feed occasionally on stems. In the laboratory, *B. laticollis* can be reared successfully on cacao shoots, but, although eggs and young nymphs have been found on stems, there is no record of this species completing its development on them in the field.

Mirid Damage.

The damage to cacao tissues which have been subject to feeding by Mirids has been described in detail by Squire (1947) and by Crowdy (1947), and the physiology of the feeding process has been investigated by Goodchild (*in press*).

S. singularis and *D. theobroma* may be regarded as typical plant-feeding Heteroptera, and, as with the Apple Capsid (Smith, 1920), damage to the host tissue is not restricted to the point of insertion of the stylets of the mouth parts. Saliva is injected into the wound and this has a marked histolytic effect, probably due to the action of esterases (Goodchild, *in press*). Whatever the actual nature of the reaction, a well-marked black lesion develops, considerably more extensive than could be caused by the simple mechanical destruction of cells by the stylets. If these lesions completely ring the shoot, it dies. Such direct killing is not as important as the subsequent invasion of lesions by the weakly pathogenic fungus, *Calonectria rigidiuscula* (Berk. & Br.) Sacc., which is associated with an acute and a chronic form of cacao dieback (Crowdy,

*Now at The Zoology Department, The University, Reading.

1947). The infected lesions show subsequently as roughened areas of bark or as cankers.

On pods, the feeding site is marked by a black plug of dead tissue and a number of these lesions on a young pod may cause distortion during growth or even death. Loss of crop through such damage is negligible, and this explains the relative unimportance of *B. laticollis* and *H. bergrothi*.

To appreciate the effects caused by feeding on stems, some knowledge of the branching habit of the cacao tree is essential, and an excellent account has been given by Cheesman (1934). The vertical axis of the seedling divides into about five horizontally displayed "fan branches" which bear their leaves in two rows. From beneath the "jorquette", or point of forking, a vertical "chupon" grows, distinguishable from a fan by its upright habit and spiral phyllotaxis. This in turn jorquettes, new chupons develop, and eventually the seedling axis and the main chupon become the trunk of the tree. New chupons arise from the base of the trunk, and in cultural practice these new shoots are generally trimmed off, though they may be allowed to grow to replace the original trunk should that fail.

The resulting picture in a mature cacao farm is of tall spindly trunks holding aloft, at about fourteen feet from the ground, a continuous canopy of interlacing branches. This sharp division into a canopy-layer and the under-canopy holds for the elements of the branch system. The canopy consists mainly of fan branches, those which arise from the jorquette branching in their turn, while new systems of the under-canopy levels are chupons, though these will jorquette below the canopy layer and provide some fans. Chupon and fan offer very different features to Mirids, the fan in general being smaller, thinner and more woody.

Vegetative growth is not steady, but is characterised by periods of rapid elongation of the stem and expansion of leaves. During this "flush", which occurs about five times a year, the stems are soft and succulent, later hardening off and becoming woody. The suitability of either stem type for Mirid feeding, therefore, varies considerably during the year.

Mirids feeding on the fan tips rapidly kill them, and the dead twigs remain hanging for some time, becoming brown as they dry. The trees present an appearance similar to that caused by burning, and to such damage the term "blast" is applied (Voelcker & West, 1940). These symptoms may be seen uniformly over several acres in January or February, but this seasonal blast has very little effect upon the well-being of the tree. More important are the smaller clumps of perennial blast, with unthrifty trees, heavily cankered and continually putting out weak flushes apart from the main flushing period. Such trees have a very characteristic appearance, the crown being much branched, but only thinly clothed with leaves; it is very aptly described as "stagheaded". This type of damage is accompanied by an obvious reduction in yield.

Finally, there is the "Mirid pocket" in which a small area of severely damaged trees stands out in sharp contrast to the surrounding healthy cacao. Normally about 50 trees are involved, the canopy branches are completely lost and the trees regenerate by chupons. These are killed by Mirid attack and fresh chupons develop which are killed in turn. This vicious circle is accompanied by the complete absence of utilisable pods and ends in the death of the tree.

The actual loss of crop due to Mirids has not been assessed, though Box (1944), by an arbitrary evaluation, suggests that it cannot be less than 20 per cent. of the annual Gold Coast production. The majority of workers familiar with West African conditions suggest that the chronic effect of Mirid attack is as serious as the much more spectacular ravages of swollen shoot. Loss through swollen shoot has been estimated (Posnette, 1947) to be at least £1 million per annum at the prices ruling in 1945, which were much lower than those of the present day.

The effect on young cacao is much easier to see. Voelcker and West (1940) record that, in the early days of the Gold Coast cacao industry, trees came into bearing after four years, and this is still true of the experimental plantings at W.A.C.R.I. where Mirids are rigorously controlled. Present native plantings do not bear before they are about ten years old, and the delay is entirely due to Mirids, either directly to their attack or indirectly to the primitive and irrational control measures adopted by the native farmers to protect their young trees. This delay is particularly serious in areas which have been extensively replanted after the removal of mature trees affected by swollen shoot.

Methods of Sampling.

The data to be presented have been obtained entirely in the field, and from observational, rather than experimental, plots. Population sampling for Mirids has proved difficult, and, despite the variety of methods listed below, the ideal technique has not been found. This difficulty is due in part to the form of the cacao tree. The canopy is beyond reach from the ground. The crowns of individual trees are interlaced with those of their neighbours to give a deep layer, the bottom of which is damaged by ladders if these are sufficiently long to permit examination of the top. Furthermore, the population sampled is small (with a maximum of a thousand to the acre), and is very patchily distributed. Three main sampling methods have been employed; these are:—

- (a) collecting and counting Mirids within reach, the canopy layer being ignored, as in the Routine Collections;
- (b) counting without collecting, ladders being used to permit examination of the whole tree, as in the Observation Plots; and
- (c) estimating damage rather than the number of bugs. (This method gives only a very rough estimate of the population, the correlation between lesions and Mirid numbers, in the single plot in which a measure of the two was made simultaneously, being low ($r=+0.668$ significant at the level $P=0.01$). Nevertheless, the method was used with advantage in the series of observation plots designated "the mealybug population plots" and in the survey of the cacao-growing areas.)

The Routine Collections give records from August 1944 to July 1948 and have been described by Nicol (1945). In this method, eight collectors walked parallel with one another through a chosen area. They collected all the Mirids found infesting trees in their path and recorded additional data such as the number of trees searched, the number of pods searched, the number of bearing trees and the state of flush of the trees. Collections were made daily (except Sundays) and, to avoid depopulation, different areas were searched on successive days. The method has obvious disadvantages; the search is effective only up to a height of about six feet, and the larger nymphs are skimmed away from a considerable area so that quick re-establishment of a balanced population is prevented. Nevertheless, it was hoped that such a simple method would show the gross fluctuations in population throughout the year, and the most striking differences between successive years.

Observation Plots.—Nine plots of up to 200 trees in each plot were marked out. The trees were individually numbered and searched, ladders being used to allow examination of all parts of the tree. The period covered was from January 1947 to June 1948 and during this time eight of the plots were examined twice a month and one plot, in a Mirid pocket, every day. In both types, the Mirids were counted, but not removed, and were recorded in detail as to instar and the part of the tree on which they occurred, with discrimination between chupon, fan, pod, and the parts, collectively, that are not included under these terms, chiefly the bark of the trunk and thicker branches. Pods were counted and flush evaluations made once a month.

As originally planned the plots, which were to be examined at two-weekly intervals, were distributed between healthy cacao (three plots) and Mirid pockets (five plots). Unfortunately, the projected comparison could not be made as one of the healthy plots was severely blasted in the first few months of the observations, and another became a Mirid pocket after being damaged by a falling shade tree which was blown down in a gale in October 1947. The remaining good plot comprised only 48 trees.

Mealybug Population Plots.—These, which have been described by Strickland (1951), were utilised by the Mirid workers to examine the canopy branches. In ten 1-acre plots, 12 trees, taken at random, were felled in each month. Chupons and fan-branches were taken at random from the felled trees and the number of fresh black lesions counted. The bark was then stripped and its inner face searched for Mirid eggs. Any eggs found were fixed in alcoholic Bouin's fixative and taken to the laboratory for identification. No count was made of Mirids as the adults could fly freely away and nymphs would be dislodged by the felling. Records were made for a period of six months only, from September 1947.

Survey of cacao-growing areas.—By the end of 1949, the plots and the cacao in general around Tafo had yielded sufficient information to suggest the course of Mirid attack and the factors of importance in connection with it. To determine whether the same interactions held over a wider field, and to give a quantitative estimate of these interactions, a survey was made of the cacao-growing areas of the Gold Coast. The base-lines were the motor roads, and sorties were made into the farms on either side. The interval between successive sorties and the length of them were determined by the distribution of the cacao in the district. At intervals a small group of trees (about 30) was taken at random and a survey-form completed for it. This form included classifications of those factors considered to be relevant: the isolation or otherwise of the stand of which the chosen group was a sample; the age and the spacing of the cacao trees; the presence or absence of green, ripe, or diseased pods; and the general condition of the cacao, together with notes on the slope and drainage of the site.

The factors analysed in greatest detail below were the intensity and nature of the Mirid damage, the shade conditions under which the cacao was growing, and the condition of the cacao canopy. The categories employed in the survey were as follows:—

Mirid damage was broadly classified into three groups, Absent; Slight, where damage could be found but the trees were not visibly set back; and Severe, where the trees were quite obviously set back. Finer distinctions were then drawn between Recent and Old damage. Recent damage was taken to include only the fresh black lesions and was classified by the site of the lesions, whether on pods, chupons, fan tips, or affecting the whole canopy. Old damage was divided into degrees of severity, from cankering only, through stagheadedness, to loss of canopy branches, with a separate category for the die-back of seedlings.

The canopy of the cacao itself was described as Complete; Complete but thin; or Incomplete, this last class being further sub-divided to show whether the canopy had not yet formed, whether the gaps were confined to patches between the crowns of adjacent trees, or whether the crown branches themselves were lost.

For shade conditions the primary division was into Low Shade, provided by trees with crowns which just surmounted those of the cacao trees; and High Shade provided by tall forest trees. Each type was then divided into the degrees, Absent; Sparse; Dense; or Very Dense. Low Shade Sparse, included all up to half cover; Dense, over half cover; Very Dense, complete cover. High Shade Sparse, included up to quarter cover; Dense, up to half cover, and Very Dense, three-quarter cover or over. After completion of the survey form the trees were searched for Mirids, but

the period of the survey (December to March) had been selected to reveal Mirid damage, and this is most obvious after the population peak.

Complete randomisation would have given an overwhelming majority of healthy cacao in some areas and of badly damaged cacao in others. Comparison of associated factors would therefore be complicated by regional differences. To compensate for this, departure from strict randomisation was made so as to include equal numbers of good, bad and indifferent stands on each day. This stratification was not made on Mirid damage but on the gross *facies* presented by the cacao.

All the counting methods have some degree of error, as the early instars are small and easily overlooked. This error is greatest in the routine collections, which are naturally somewhat cursory, so that few of the young nymphs are collected. The error is at a minimum in the daily observation plot and here the workers achieved a very high standard. In this plot, nearly all the individuals that occur on the trees are detected, if they remain longer than one day, and their life histories can be followed. It is remarkable that 15 per cent. of the *D. theobroma* present were first seen in the egg stage, the only sign of which is a pair of slender filaments, about 1 mm. long, projecting from the bark, 64 per cent. were seen in the small first instar, and 13 per cent. in the second instar. The remaining 8 per cent. were found in later instars, but these were confined to trees too tall to be thoroughly searched, even from 14-ft. ladders, and probably represent migrants from the part of the trunk beyond reach.

The eggs and earlier instars of *S. singularis* are less conspicuous, and for this species only 3 per cent. were found as eggs, 61 per cent. as first instar nymphs, and 22 per cent. as second instar nymphs.

Factors associated with Mirid Damage.

In the field, the most conspicuous feature of Mirid attack is its patchy distribution, stands which are otherwise healthy showing small groups of severely attacked trees. This character is probably not due to the action of the Mirids themselves, as it is also shown by a number of cacao diseases (Crowdy, 1947).

Mirid damage is to be found on all the principal soil types occurring in the Gold Coast (Anon., 1947), so that soil differences are not the primary cause of such a distribution. Poor soil and bad drainage inevitably have an adverse effect upon the recovery of attacked cacao.

The amount of shade provided for the cacao is an obvious factor to investigate and this has been done in part. Posnette (1943) has shown from observations on 1/16-acre plots that the *total amount* of Mirid damage is greater for unshaded plots but that *severe damage* was particularly associated with the presence of overhead shade. Squire (1947) records that pockets are associated with cultivation under shade trees and that in regions such as Nigeria, where cacao is grown without shade, damage takes a more diffuse form.

The survey of cacao-growing areas permits a detailed examination of the factors operating in the damaged local patches and in the surrounding healthy cacao. But the analysis of the results is open to two dangers, since the selection of survey areas was not entirely at random. The possibility of spurious correlations is increased; and strong correlations working in one direction may mask others, equally real, which work in the opposite direction. To make the maximum use of the data while avoiding these pitfalls, the following method of analysis has been used.

Two-way tables were prepared against intensity of Mirid damage for each factor observed in the survey. Records of all of the 1,081 stations were included in these tables and the χ^2 test was used to determine which factors were significantly associated with damage. Tests were also carried out for all pairs of the factors which were found

to be associated with damage to determine the association with each other. In this way the "main effects" were sorted out. Sub-samples were then selected, homogeneous for main effects, and those conditions which previously had not proved significant were re-tested against Mirid damage and each other. This was repeated until all factors were shown definitely to have some effect or no effect and, with the various interactions ascertained, amalgamation of the results was possible so as to give an unbiased picture yet retaining adequate numbers in the individual cells of a contingency table.

TABLE I.

Distribution of Mirid damage with respect to shade and the state of the cacao canopy. (Each frequency is expressed as two percentages, one summing vertically and the other horizontally.)

Low Shade	Canopy	Mirid Damage			Total
		Absent	Slight	Severe	
Absent or Sparse	Complete	66 89	8 10	1 1	100
	Thin	16 41	16 39	6 20	100
	Broken	18 9	76 33	93 58	100
	TOTAL	100	100	100	
Dense or Very Dense	Complete	54 90	8 9	1 1	100
	Thin	40 47	52 42	12 11	100
	Broken	6 6	40 27	87 67	100
	TOTAL	100	100	100	

In the preliminary examination, the different levels of Mirid damage were associated with only two factors, the density of low shade (*i.e.*, shade provided by trees which just top the cacao canopy) and the completeness or otherwise of the cacao canopy. The relationship is summarised in Table I. In the compilation of this Table, all of the 1,081 stations have been used, but the frequency in each cell has been expressed as a percentage of the marginal totals, first vertically and then horizontally.

The figures that add up to 100 horizontally demonstrate clearly the effect of canopy condition on Mirid attack. Attack is more prevalent where the cacao canopy is incomplete and this is true under all shade conditions. Thus, when the canopy is complete, 89 per cent. and 90 per cent. of the stations are free from attack, whereas in areas with broken canopies the percentages free from attack fall to 9 and 6, respectively. The pairs of figures are necessitated by the presence of two shade classes. This association is in fact so close that it has made further subdivision very difficult.

The only direct effect of shade on Mirid damage is the increase in the severity of damage under dense shade; this rises to 67 per cent., as compared with 58 per cent. under sparse shade when the canopy is broken. This difference agrees with the observations of Posnette, noted above.

The striking feature of the figures that sum vertically is the effect of shade intensity upon the nature of the cacao canopy. Dense and very dense shade delays the formation of a canopy in young cacao, and even when formed, the canopy remains thin. Thus, for the three degrees of Mirid attack, the percentage of stations with canopies that are thin or not formed increases from 16 to 40, 16 to 52, and 6 to 12, respectively, for the two shade classes.

Cacao farms are generally shaded, not only by trees whose crowns immediately surmount the tops of the cacao, but also by a variety of forest trees which may be as tall as 200 feet. This type of shade has some, albeit slight, effect upon the cacao canopy, as is suggested by an analysis of the 376 stations where there was no Mirid attack. Table II shows this relationship, the frequencies being expressed as percentages adding up horizontally. The effects of different degrees of high shade upon the canopy differ with different amounts of low shade. When there is no shade or only sparse shade provided by the shorter trees, increasing intensities of shade by high forest trees tends to increase the proportion of complete canopies beneath. This effect is exerted by reducing the frequency of broken canopies rather than by any alteration of the number of thin canopies, and is due to the mitigation of exposure conditions which, unchecked, rapidly lead to degradation of the cacao with a die-back of the crown.

TABLE II.

Effect of shade conditions upon the state of the cacao canopy when there is no Mirid damage.

(The frequency of each class of canopy is expressed as a percentage for each shade condition.)

Low Shade	High Shade	Cacao Canopy			Total
		Complete	Thin	Broken	
Absent or Sparse	Absent	51	10	39	100
	Sparse	71	19	10	100
	Dense	74	17	9	100
Dense or Very Dense	Absent	55	31	14	100
	Sparse	50	47	3	100
	Dense	61	39	0	100

Cacao already heavily shaded by short trees is shielded from exposure damage, and the effect of high shade is not so marked, apart from a slight tendency to reinforce the etiolating effect of the low shade.

These 376 stations are useful in evaluating the optimum conditions necessary for an intact canopy as they are not complicated by the effect of Mirids. Apart from the shade conditions, they may be used to determine the effect of age and of spacing, but the results are not presented in tabular form as they are self evident. For any given age of cacao stand (as estimated by the girth of the trees), the completeness of the canopy is inversely proportional to the spacing, while at any given spacing the older trees have the more intact canopies. One interesting modification of this general rule is exhibited by stands of trees estimated to be over 30 years old. These were present at 64 stations altogether; at 45 of these the canopies were complete, at 16 they were broken and at three only were the canopies thin. This peculiarity cannot

be explained by any combination of factors such as spacing or shade, and it is suggested that if degradation once starts in these older stands the process is very quick and the intervening stages between healthy and badly degraded are very transient.

Finally, the 401 stations at which damage was severe show the influence of shade upon the nature of damage. Severe damage may be analysed from the survey records into three types; these are the presence of severe cankering due to invasion by *C. rigidiuscula*, the stagheaded condition and the Mirid pocket with loss of crown branches. Only 28 stations cannot be so classified from the field notes and the remaining 373 were approximately equally distributed among the three shade classes (low shade absent, 136; low shade sparse, 124; and low shade dense or very dense, 113). The relative distribution of the stations in each shade category with respect to type of Mirid damage is shown in Table III. Pockets are more typical of cacao grown under dense shade, whereas in the more open conditions, designated "low shade sparse or absent" in Table III, the stagheaded form is more prevalent. Thus, within the boundaries of the Gold Coast, the general statement of Squire (1947) regarding the distribution of the different forms of damage in West Africa is found to be true.

TABLE III.

The effect of different degrees of low shade upon the nature of severe Mirid damage. (The frequencies of the types of damage are expressed as percentages for each condition of shade.)

Low Shade	Type of Damage			Total
	Cankers	Staghead	Pocket	
Absent 	16	51	33	100
Sparse 	19	37	44	100
Dense or Very Dense	25	25	50	100

The bearing of these factors, together with some others which were noted qualitatively rather than quantitatively, upon the course of Mirid attack may be summarised as follows.

The type of cacao grown in the Gold Coast, West African Amelonado, is not a particularly robust tree and tends to a tall stemmy growth. This tendency is exaggerated under dense shade, particularly dense low shade, by too close spacing, or by cultivation in waterlogged soil. The result is a spindly, etiolated tree in which the balance of vegetative growth is upward by chupons rather than laterally by fans. The crowns of individual trees remain small and the canopy of the stand thin. If the canopy is broken, regeneration is by chupons and these provide ideal breeding sites for Mirids. A large population is built up which rapidly kills the growing chupons and the trees are reduced to trunks clothed by attacked chupons. In this way, the typical pocket, associated with shade, is formed.

Under conditions of reduced shade, and with a wider spacing, lateral spread of the trees is encouraged, the vegetative balance is predominantly fan, and the final canopy is a thick layer. Should this canopy now be broken, regeneration is by fans. The new fans provide feeding material for Mirids, but not a breeding site, so that the subsequent damage is more diffuse. Unfortunately, the cacao so opened up is not protected by overhead shade. The trees suffer from exposure, and the effect of this, coupled with Mirid attack, is a degeneration of the canopy. The fan proliferations are quickly killed and stagheaded cacao, more commonly associated with unshaded cacao, is formed.

The formation of a pocket is not impossible under reduced shade. Die-back due to *C. rigidiuscula* may lead to the complete loss of crown branches and the trees are reduced to poles. But such a state only follows after the staghead stage and so it is not nearly as common as under shade conditions. Not only is the genesis of the two types of pocket very different but also the subsequent fate. The shaded pocket remains circumscribed while the unshaded pocket spreads outward. Loss of the crowns over-exposes another ring of trees which similarly deteriorates and exposes a further ring. The culmination of this process was observed in a young Ashanti farm where, by interaction of Mirid attack and exposure, the trees were reduced to mere trunks over an area of an eighth of a square mile.

Cause of the Initial Break in the Canopy.

The above summary traces the degradation of cacao after the canopy is broken. There remains the problem of the cause of the initial break, whether it may be due to Mirids alone or to other factors. Particular attention was paid to this aspect at the survey stations with broken canopies, and although in very bad cacao such an evaluation must be subjective to some extent, it was obviously true that at 70 per cent. of the stations the original break was due to factors other than Mirid attack.

Thus, for 49 of the samples, no Mirid invasion had taken place at all, and for a further 37, the trees had obviously been damaged by falling shade trees. The most common causes of breaks were the die-backs associated either with swollen shoot (178 stations) or with adverse water relations (101 stations), this latter term including drought, waterlogged soil and a rapidly fluctuating water table. After the exclusion of a few miscellaneous causes (*e.g.*, lightning), only 185 of the original 605 stations could not clearly be shown to have a cause other than Mirid attack. For these, the insects had either initiated the damage, or the extent of their subsequent invasion had completely obliterated signs of the first cause; this second explanation is certainly true of some, for in the early stages of the survey it was not appreciated that waterlogged soil had such a profound effect upon the growth habit of the cacao tree, making it susceptible to attack. Field experiments, in which an attempt was made artificially to cause a pocket, suggest that this masking effect is operating in the majority of the 185 stations, though it is impossible to prove this contention.

Pockets may be induced experimentally by breaking the canopy. In 1946, seven plots were selected, the layout being determined by the areas of cacao available on the Institute's land and not by any statistical criteria. Two plots, C_1 of 44 trees and C_2 of 45 trees, were left as untreated controls. In three other plots, the canopy was damaged mechanically, either by cutting the crown branches back to the main trunk (Plot P_1 of 31 trees) or by pollarding at a height of five or six feet from the ground (P_2 of 37 trees and P_3 of 25 trees). The trees on the remaining two plots (AC_1 and AC_2) were colonised with Mirids obtained from the routine collections; for AC_1 , 3,161 were placed over 20 trees during the period June to November, and for AC_2 , 2,459 were distributed over 24 trees during the shorter period, September to November. *S. singularis* and *D. theobroma* were used on both, the number of each depending entirely on the number of viable bugs brought in by the collectors. *S. singularis* comprised 61 per cent. of the Mirids liberated in AC_1 and 69 per cent. in AC_2 .

From January 1947 to July 1948, inclusive, weekly counts of the number of bugs present in each plot were made. Subsequently the plots were visited regularly to note their progress, though no counts were made on these visits.

The immediate histories of the treated plots were similar, damage to the crown being followed by vigorous chupon regeneration. The plots were invaded by Mirids in December 1946, when the population in the surrounding cacao was increasing. P_1 had been pollarded in May and chupons were present in June but, even here,

invasion did not take place until December. For the period of weekly counts, the mean total numbers per calendar month of Mirids seen in the control plots were 3 and 1, respectively, while the totals in the treated plots were consistently higher; the mean numbers of Mirids per count were only 0.7 and 0.2 respectively for the control plots, and were consistently higher for the treated plots, *viz.* $P_1 = 35.4$, $P_2 = 30.6$, $P_3 = 18.6$, $AC_1 = 46.3$, and $AC_2 = 14.2$. Breaking the canopy by either agency therefore leads to the formation of a focus of infestation.

The later histories of these plots show that, in the maintenance of a typical pocket, aggravating factors, especially exposure, are of great importance. By July 1948, P_1 had completely recovered although it had supported a larger number of Mirids during the first peak period than any other plot. P_2 was in marked contrast at this later date with 11 trees dead, 9 dying and the remainder very obviously set back. Numbers of Mirids are not the cause of the difference, which appears to be due more to the relative exposure of the two plots. P_1 was well shaded above by forest trees and at the margin by the surrounding healthy cacao. As the trunks were intact to their original height, and the early phases of regeneration were not hampered by Mirid invasion, the hole in the canopy was quickly plugged. There was no overhead shade for P_2 and marginal shade was absent in the south corner. The concentric arrangement of the different degrees of damage is shown in fig. 1, which is a plan of the plot. The central and southern trees are dead; these are surrounded by a ring of dying trees which have received slight protection by the marginal wall of cacao. The outer trees are pushing out some lateral growth and are the healthiest of the treated trees and these are comparatively well shaded by the surrounding cacao.

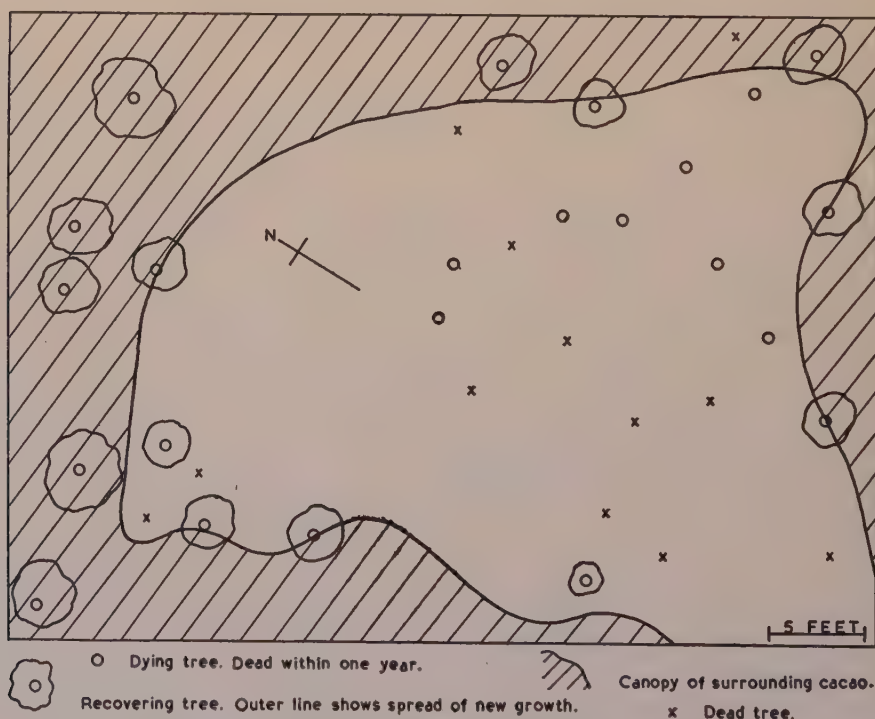


Fig. 1.—Plan of pollarded plot, P_2 , showing the condition of the trees two years after treatment.

As with the difference between plots P_1 and P_2 , so the differences within P_2 cannot be explained by intensity of Mirid attack. The first deaths of trees were in November 1947, and up to that date the mean number of Mirids per tree was 123 for those which subsequently died, 127 for those subsequently classed as dying and 130 for the remainder. Although the means are so similar, the individual counts per tree were examined by the analysis of variance. For the analysis, the square roots of the individual counts were used (Upholt, 1942) as the bugs show a contagious distribution. The analysis revealed no significant difference in the number of bugs among the three levels of damage.

If subsequent exposure is a main factor in the continuation of a Mirid pocket, the size of the hole in the canopy will determine the fate of a patch of damaged cacao. This is found to be so in the field, pockets not being formed where the gaps are less than about 600 sq. ft. in extent. In the survey, 37 of the pockets had been initiated by fallen trees. The tree most commonly felled in the cacao belt is probably the oil palm (*Elaeis guineensis* Jacq.) which is then tapped for "palm wine". The mature palm reaches above the crowns of the cacao and in falling does some damage to the canopy, but the felling of a single palm has never been seen to lead to the development of a pocket. At one of the survey stations, a clump of four palms had been felled, fan-wise, so that the damage exceeded 600 sq. ft. and a flourishing pocket was in the process of establishment.

Of the artificially colonised plots, AC_2 suffered a temporary set-back but had recovered completely within a year, showing its history of damage only in the severe cankering of the branches. AC_1 , under the same shade conditions as AC_2 , but with a larger initial gap in the canopy after treatment was concluded, continued as a pocket. Here, as in P_2 , the outer ring of treated trees was making good recovery in July 1948 and the Mirids were concentrated on the central exposed trees. This is an example of a pocket started initially by Mirids and maintained by the usual combination of Mirid attack, invasion by *C. rigidiuscula*, and exposure.

The essential condition for the initiation of a pocket of Mirids is that a large number should attack contiguous trees; the immediate damage is then sufficiently extensive to cause the continuation of the pocket. This was achieved experimentally in AC_2 , but it is important to know whether it could have been achieved under ordinary field conditions in a stand of uniformly healthy cacao. The answer to this question is not merely of academic importance, but is vital to the formulation of cultural control methods. If Mirids alone cannot cause sufficiently large breaks, then the problem of prevention of damage by them becomes one of maintaining a good canopy.

If cacao is grown without shade, then the first attacks of Mirids may be the immediate cause of degradation, as in much of the Nigerian cacao. Even seasonal blast will open up the canopy at a time when the effect of exposure is coming to its maximum, *i.e.*, at the beginning of the dry season, and the chronic stagheaded condition of much of the Nigerian cacao is the result. In the Gold Coast, cultivation of cacao will, presumably, continue to utilise shade trees, and under these conditions lightly blasted cacao will be shielded from exposure effects and will recover. Absolute proof that the insects themselves do not start pockets is lacking, but it seems unlikely that this is so because of three factors: the actual distribution of the bugs in the field, their distribution over the tree, and their breeding and feeding preferences. The extent to which each of these is relevant to the problem is considered below.

Distribution in the Field.

The distribution of Mirids over uniform cacao was estimated at Adonkwanta, an area planted by the Institute but not on its land at Tafo. A plot with four sides, each 3 chains long, was marked out among seedling cacao which had been

planted at stake, three seeds per picket, in rows spaced 5×5 ft. The number of bugs per picket was counted in December 1949 and subsequently at monthly intervals.

The total population was low, with 75 *D. theobroma* and 70 *S. singularis* on the 1,088 pickets in December, suggesting that a Poisson distribution would fit that observed. But this was not the case, nor could a fit be established for any of the counts. From the records of the daily observation plot, it was clear that the gravid female laid eggs in groups of up to seven at a time, so that the theoretical distribution tested must include the random distribution of groups rather than individuals. A very good fit was obtained with the negative binomial distribution. In the computation, the mean, m , was taken to be the observed mean, and the parameter, k , calculated from the formula $P_0 = (1 + m/k)^{-k}$, where P_0 is the probability of finding plants with no Mirids and, like m , was determined from the field data. Using these parameters, the calculated frequency distribution is shown in Table IV, and is compared with the observed frequency distribution. The fit is very close. When examined by the χ^2 test, the values of P for *S. singularis* and for *D. theobroma* were $P = 0.80-0.70$ and $P = 0.98-0.95$, respectively.

TABLE IV.

The observed frequency distribution of Mirids over 1,088 seedlings compared with the theoretical distribution calculated from a negative binomial.

Number of <i>S. singularis</i> per seedling	Observed	Calculated	Number of <i>D. theobroma</i> per seedling	Observed	Calculated
0	1,060	1,061	0	1,026	1,026
1	9	10	1	36	37
2	4	5	2	15	13
3+	15	12	3	5	6
			4+	6	6

The data from the observation plots were similarly tested, for both healthy and damaged cacao, and, for each, at a time of high population (December) and of low population (June). In no instance did the observed distribution depart significantly from a negative binomial, so it may be concluded that in uniform cacao the distribution of Mirids is the result of random dispersal of egg clutches of varying sizes. It is difficult to see how such a distribution in cacao, originally healthy, could give a sufficiently large number of bugs on contiguous trees to cause a gap in the canopy of some 600 sq. ft. In the normal course of establishment certain trees must be already more favourable for Mirids than the area in general. On these trees Mirids would become established and, as the population increased and the trees became severely damaged, the adults would fly away in search of favourable trees.

This contention is supported by certain field observations. The daily observation plot was in a pocket that was rapidly being killed out by the large population of Mirids present, but at no time did the insects spread to the surrounding healthy trees. Pollarded plot P_2 was then sited 20 yards to the south and, when chupon development started, invasion by Mirids immediately took place. The source of invasion was obviously the daily observation plot, as this plot was unusual in the high proportion of *D. theobroma*, a preponderance reflected in the Mirid population invading plot P_2 . Even with established foci of infection on two sides, the intervening belt of healthy cacao remained unattacked.

Distribution over the Trees.

Although the number of Mirids counted is normally low, it is conceivable that this small population could cause a break in the continuity of the canopy if it were

concentrated in that layer. Unfortunately, the counts made in the observation plots do not record the distribution of the insects with reference to the canopy, but by their presence on fans or on chupons. This does give some measure of the distribution, as fans are predominant in the canopy and chupons below. However, a direct count was made in a single observation plot, after the regular observations were suspended, to cover the period August 1948 to March 1949, at fortnightly intervals. This period includes the time of maximum numbers of Mirids.

Direct counts of the Mirids within and beneath the canopy were possible in this plot as the canopy was sufficiently degraded to permit the use of long ladders, but not so degraded as to be virtually lacking. As this type of cacao sends out many chupons at the crown, it must be emphasised that the counts will be higher than those made in really good canopies. Even so, only 284 Mirids, representing 20 per cent. of the number observed during the period of the fortnightly counts, were within the canopy layer. As more pods were present beneath the canopy than within it the number of Mirids on pods may be discounted by considering only the population on stems. This attained its maximum during January and February, with a total of 347 for the four counts made during the period. Of this total only 37 were observed within the canopy.

In fact, the bugs within the canopy layer appeared to be a diffuse feeding population, mainly adult, maintained only by migration from the population breeding on pods at a lower level. The population on pods became extinct in February and then the canopy population fell to its minimum, while up to that time the two populations were strongly correlated ($r=+0.84$, significant at the level $P=0.01$). As pods dwindled in importance during the harvesting period, the Mirids moved to the stems at the sub-canopy level so that this population attained a maximum in February, and up to that date there was a strong negative correlation ($r=-0.93$, significant at $P=0.01$) between the numbers on pods and the numbers on stems. This increase in the stem population, which has been found every year in the routine collections, had been thought to reflect a change in the higher layers, but in this plot, where direct counts of the two were made, it was obviously not so. Further work is needed on this point as "seasonal blast" certainly suggests that the canopy population is augmented at the beginning of the dry season, though it may be that the importance of Mirids in the aetiology of seasonal blast has been over-emphasised.

Feeding and Breeding Preferences.

In the mealybug population plots, counts of lesions gave an estimate of the amount of Mirid feeding and counts of eggs the amount of breeding. As the canopy stems are chiefly fan and the sub-canopy stems chupon, this gives an indirect evaluation of the two sites which may be used in cacao that is not amenable to the direct counting technique.

Altogether, 648 chupons and 2,855 fans were examined and of these 60 and 54 per cent., respectively, showed some signs of feeding, while, for more intense feeding (6 lesions or more), the difference was even greater—33 and 19 per cent., respectively. In 2×2 contingency tables, these figures give values of χ^2 significant at $P=0.01$, showing that fans are less favourable feeding sites.

This difference may be due to the physical differences between the two types of stem, or it may be a reflection of the fact that fans occur in a continuous stratum while chupons are more in isolation. It is possible to test the relative importance of these differences as only a half of the plots had intact canopies. If spatial arrangement is the determining factor, the intensity of feeding on fans in these plots should be different from that in the other five.

A common sub-sample of 30 fans was taken at random from each plot for each month and an analysis of variance performed for plots against months. The greater

part of the variation proved to be due to differences between months, and the difference between plots was not significant. It is the fan itself, therefore, which is unfavourable for feeding and not the arrangement of fans in a continuous canopy.

Eggs were found on only 15 occasions; although more than four times as many fans as chupons were searched, no eggs were found on fans. Fans, therefore, appear to be a negligible breeding site for Mirids although subject to a low intensity of feeding, and this confirms the disparity, noted above, between the numbers of Mirids found within and beneath the canopy.

Finally, if the above statement is true, there should be a difference between the adult/nymph ratios over the various parts of the tree. At a breeding site, a gravid female will give rise to nymphs and will give a low ratio, while at a feeding site adults will be relatively more common. In the observation plots, counts were made of adults and nymphs, and for the plots in Mirid pockets the percentages of adults on the different parts of the tree were as follows:—

Pods	7.4	(based on a total count of 4,960 <i>S. singularis</i>)
Chupons	13.5	(„ „ „ 7,646 „)
Fans	19.1	(„ „ „ 2,315 „)
Bark	37.4	(„ „ „ 626 „)

The totals represent the amalgamation of fortnightly counts in five plots, covering a total period of 18 months, so there is ample opportunity within these gross results for inconsistency. A preliminary analysis showed that there were no consistent differences between plots over the experimental period but that there were considerable differences between months. A final analysis was therefore carried out on the totals for individual months. The method of analysis employed was that of fitting constants (Yates, 1934), *a* constants for parts of plant and *b* constants for months. The final analysis is shown in Table V. Differences in the percentage of adults between months are significant (at $P=0.01$), as would be expected from previous observations that there are eight fairly distinct generations per annum. More pertinent here is the finding that the differences in the proportion of adults

TABLE V.

Analysis of variance of the percentage adult *S. singularis* on chupons, fans, pods, and bark for 18 months.

	degrees of freedom	sums of squares	variance	F.
<i>Test for parts of plant.</i> (<i>a</i> constants)				
Months only ...	17	270,818.7	78,434.17	42.76
Parts of plant ...	3	235,302.5		
TOTAL ...	20	506,121.2		
<i>Test for months.</i> (<i>b</i> constants)				
Parts of plant only	3	382,300.8	7,283.55	3.97
Months ...	17	123,820.4		
TOTAL ...	20	506,121.2		
Reduction due to fitting constants ...	20	506,121.2	1,834.47	
Interactions ...	44	80,716.7		
TOTAL BETWEEN CLASSES	64	586,837.9		

in the different parts of the tree are highly significant (at $P=0.001$), showing that there are consistent differences throughout the period of examination.

The evidence, although mostly indirect, suggests that Mirids exploit areas where the canopy is already broken rather than make such breaks themselves. The major part of the population is found beneath the canopy layer, as this layer is unsuitable for Mirid development. The diffuse feeding to which the canopy twigs are subjected does not set back the hardened fans as these are tough and woody. Flush tips may be killed, but such attack has little effect unless the tree is inadequately shaded, or unless the conditions of growth favour the establishment of *C. rigidiuscula*.

Conditions determining Colonisation.

Once suitable conditions are available, invasion by Mirids is very thorough, for only 8 per cent. of those stations (sampled in the survey) which were suitable for the insects were free from attack. There must, of course, be a sufficient population to invade; patches of cacao damaged during the period of population minima may escape. Falling trees are the most spectacular of the damaging agencies, but it is often observed in the field that damage caused in this way during March or April, when the number of bugs is extremely low, escapes subsequent exploitation. The treated plot P_1 , for example, remained free of attack during the period June–November, although eminently suitable for colonisation. The plots treated later were immediately invaded; similarly, in the healthy observation plot damaged by a tree blown down in a gale in October 1947, the number of *D. theobroma* increased from an average of 5 per 100 trees to 43 in one month, in contrast to the comparable healthy plot where the number continued at the old level of 5 per 100 trees.

Given an adequate population, the establishment of a breeding focus in damaged sites may be due either to random wandering of adults, followed by a build-up where material suitable for breeding is abundant, or to definite movement into the area. The stimulus controlling migration may be provided by the presence of suitable chupons, or by the resulting change in physical factors, such as an increase in light intensity.

Against the theory of random movement, it should be pointed out that although developing pods form an ideal breeding material, Mirids are not spread uniformly over the pods borne by healthy cacao, even where such trees are adjacent to an abundant population of Mirids such as that provided by a pocket. To test experimentally the factors at work, an extension of the artificial initiation of pockets was made in November 1949. The lay-out of the experiments was in three randomised blocks of three plots, the treatments in each block being:

(a) Pollarding at a height of about 4 ft. from the ground;

(b) as for (a) but with artificially-provided shade erected over the plot after treatment. For this a matting was made, similar to the palm-matting used in cacao-drying except that alternate laths were omitted, so giving about $\frac{1}{2}$ -in. slits separated by $\frac{1}{2}$ -in. ribs. The mats were laid on a framework of wire 8 ft. above ground level.

(c) Untreated controls.

Each block was in well shaded cacao to avoid the complications of over-exposure. Each plot was exactly 30 ft. square, area being used as the basis of enumeration as the individual trees varied in size. If the smaller trees regenerated less prolifically than the larger trees, but were more numerous on a given area, the result would be a comparable amount of chupon material. In practice, this was achieved quite well by selecting blocks where the canopy was originally of uniform density.

Treatments were completed in November 1949 and chupons were present by the middle of December. From the 28th of that month weekly counts were made of the numbers of Mirids and the numbers of new chupons more than 2 ins. long. The results presented are the totals to March 31st, 1950, when the writer left the Colony. Table VI summarises the results up to this final date, showing the number of trees in each plot, the total number of chupons which had developed, and the totals of the weekly counts. The figures appear quite conclusive without statistical analysis, although the lay-out permits such a treatment. The total Mirid count was higher in the unshaded than in the shaded plots and this was quite consistent for the three blocks. If the early counts only are considered, the same numerical superiority in the unshaded plots is equally evident. The difference in number of bugs was not due to a difference in the number of chupons, nor was there any obvious difference between the appearance of the chupons in shaded and unshaded plots.

TABLE VI.

Experimental initiation of Mirid pockets by pollarding. (Number of trees per plot, and total counts of Mirids and chupons, for the period December/March, in each plot.)

Block	Treatment	No. of trees	No. of Mirids	No. of chupons
D ₂	Unshaded	28	24	264
	Shaded	25	0	275
	Control	23	0	23
D ₃	Unshaded	18	85	145
	Shaded	22	6	232
	Control	21	7	18
G	Unshaded	24	446	274
	Shaded	35	191	235
	Control	29	32	11

Invasion, therefore, is a response to factors associated with breaking of the canopy other than the mere provision of breeding sites. The actual factors at work need to be determined experimentally; the most obvious difference between treatments (a) and (b) is in light intensity, though temperature, humidity, etc., will also be different.

The detailed course of population build-up could not be followed in this experiment. The time of the appearance of the first lesions did not differ consistently between the two treatments, and this suggests that there was considerable random movement of adults over all the plots. The first appearance of nymphs was much earlier in the unshaded plots than in the others, and it is suggested, as a hypothesis for future work, that the gravid female responds positively to light.

Control Measures.

The only method of Mirid "control" employed by the cacao farmer in the Gold Coast is to allow his farm to return to "bush" after planting, *i.e.*, the natural growth is allowed to spring up without check. The young cacao plants certainly remain free from Mirids during this period, but the time of first bearing is very much delayed. Moreover, when the surrounding bush is eventually cleared, the cacao trees are in a condition ideal for Mirids, being long and stemmy with no vestige of a canopy. The only result of this treatment, therefore, is to postpone the inevitable attack.

By the use of insecticides with residual toxicity, control of Mirids in seedling cacao is not only economically feasible but extremely simple (Anon., 1948), and simplicity is a necessity when one is dealing with illiterate farmers holding only small lots. The technique is to paint the stems with an emulsion containing 2.5 per cent. DDT, paying particular attention to the jorquette and to the areas of roughened bark where the nymphs rest and hide. Unfortunately, this type of treatment becomes impossible with the continued growth of the tree, so that in the present work, attention has been concentrated on older stands for which there are, as yet, no control measures.

Chemical control in older trees seems quite feasible, however, as there is no need to waste insecticide and water on the canopy. The population to be reduced is below the canopy layer and a residual insecticide applied only to the breeding sites should be effective. This reduces the task to spraying the developing green pods and the chupons.

The cultural requirements for control in no way clash with the optimum conditions for cacao cultivation. The aim of the grower should be the establishment of a complete canopy by the time the plants are too big to be painted with DDT, and the maintenance of this canopy afterwards.

In the Gold Coast, with the present types of cacao, the utilisation of shade trees should be continued. Without shade, degradation follows any set-back and under such conditions Mirids may cause the initial set-back. Shade provided by the shorter trees should be sparse so that it does not have an etiolating effect upon the cacao. Protection from exposure should be augmented by high forest trees as such shade is seldom associated with etiolation.

Even the longest-lived shade trees have to be removed at some time and, even with the most careful treatment, some damage to the cacao canopy is bound to ensue. But if damage occurs in March/April there is every prospect of recovery, while corresponding damage in November/January leads almost inevitably to Mirid attack.

Finally, cacao should not be grown where the water table fluctuates violently or where the soil is waterlogged. If, for economic reasons, such land must be planted, particular attention should be paid to these sites when applying insecticides as they are liable to become foci of infection.

Summary.

The two species of Mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.), are important pests of cacao (*Theobroma cacao* L.) in the Gold Coast. The damage sustained by the cacao is due, in part, to the direct effects of feeding by these insects, but more to the subsequent invasion of the resulting lesions by the weakly pathogenic fungus, *Calonectria rigidiuscula* (Berk. & Br.) Sacc.

In the field, Mirid damage may be classified into three categories. "Blast" is the result of light diffuse attack and is so named because of its similarity to fire damage. "Stagheaded cacao" is more severe, the trees showing numerous small crown branches but forming a poor canopy. "Mirid pocket" describes severe damage, normally limited to a small area, the trees losing the crown completely and the pole-like trunks bearing numerous lateral chupons. In general, stagheaded symptoms are more prevalent where cacao is grown without shade, and pockets where cultivation of cacao utilises shade trees.

Consideration of the factors associated with Mirid damage shows that it is correlated with breaks in the cacao canopy. Evidence is brought forward to suggest that such breaks normally precede, rather than result from, Mirid attack. The canopy itself is unsuitable for the development of either species, the major part

of the population being confined to the sub-canopy levels. The initial causes of the breaks are, most frequently, the die-backs associated with swollen shoot or with adverse water relations. The falling of shade trees causes a number of breaks, and, where cacao is grown without shade, the Mirids themselves may be a cause.

The most important single factor influencing the form of the cacao canopy, and thus the course of Mirid attack, is the amount of overhead shade, particularly that provided by trees little taller than the cacao itself. Shade which is too dense causes etiolation of the cacao and thus renders it susceptible to attack, the resulting damage generally taking the form of a pocket. Shade which is too sparse does not shield the cacao from the adverse effects of exposure. The Mirid damage in such areas generally assumes the stagheaded form.

It is suggested, from field experiments, that the invasion by Mirids of areas suitable for colonisation is not by random movements, but is determined by the change in some physical factor resulting from the broken canopy. Changes in light intensity are the most obvious results of such breaks, but further experiments are needed to determine which is the operating factor.

Degraded cacao is prevented from recovery, not by Mirid attack alone, but by the interaction of it with the presence of *C. rigidiuscula*, and by increased exposure.

The sampling methods employed are described, and the bearing of the results upon control measures is discussed.

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References.

- ANON. (1947). West African Cacao Research Institute, Annual Report. April 1945–March 1946.
- ANON. (1948). *Ibid.*, April 1946–March 1947.
- BOX, H. E. (1944). The *Sahlbergella* menace to Gold Coast cacao.—Memor. cent. Cacao Res. Sta., Tafo, no. 9, 8 pp.
- CHEESMAN, E. E. (1934). Vegetative Propagation of Cacao.—Emp. J. exp. Agric., **2**, pp. 40–50.
- CROWDY, S. H. (1947). Observations on the pathogenicity of *Calonectria rigidiuscula* (Berk. & Br.) Sacc. on *Theobroma cacao* L.—Ann. appl. Biol., **34**, pp. 45–59.
- NICOL, J. (1945). The present position of research on Caspid pests of cacao in West Africa.—Rep. Cocoa Res. Conf. 1945, pp. 111–113.
- POSNETTE, A. F. (1943). Botany.—Rep. cent. Cocoa Res. Sta., Tafo, 1938–42, pp. 19–30.
- POSNETTE, A. F. (1947). Virus diseases of cacao in West Africa. I. Cacao viruses 1A, 1B, 1C and 1D.—Ann. appl. Biol., **34**, pp. 388–402.
- SMITH, K. M. (1920). Investigation of the nature and cause of the damage to plant tissue resulting from the feeding of Capsid bugs.—Ann. appl. Biol., **7**, pp. 40–55.

- SQUIRE, F. A. (1947). On the economic importance of the Capsidae in the Guinean Region.—*Rev. Ent., Rio. de J.*, **18**, pp. 219–247.
- STRICKLAND, A. H. (1951). The entomology of swollen shoot of cacao. II.—*Bull. ent. Res.*, **42**, pp. 65–103.
- UPHOLT, W. M. (1942). The use of the square-root transformation and analysis of variance with contagious distributions.—*J. econ. Ent.*, **35**, pp. 536–543.
- VOELCKER, O. J. (1948). The West African Cacao Research Institute.—*Nature, Lond.*, **161**, p. 117.
- VOELCKER, O. J. & WEST, J. (1940). Cacao die-back.—*Trop. Agriculture Trin.*, **17**, pp. 27–31.
- YATES, F. (1934). The analysis of multiple classifications with unequal numbers in the different classes.—*J. Amer. statist. Ass.*, **1934**, pp. 60–66.
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THE RELATIONSHIP BETWEEN THE STAGE OF DEVELOPMENT AND
SUSCEPTIBILITY TO DDT AND THE PYRETHRINS OF *DIATARAXIA*
OLERACEA (L.), *TENEBRIO MOLITOR* L., AND *PERIPLANETA*
AMERICANA (L.).

By T. D. MUKERJEA.

*Department of Insecticides and Fungicides, Rothamsted Experimental Station,
Harpenden.*

(PLATES III AND IV.)

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When it is stated that a compound is toxic to a particular insect species, it usually means that the insecticide has been proved toxic to a particular stage in the life-cycle which can be conveniently treated in the field, using commercial methods of application. Little is known of the extent of variation in susceptibility of the different stages in the life-cycle.

While it is clearly recognised that there may be a qualitative difference between the toxicity of an insecticide to the egg and the active stages of a given species, it is perhaps not so clearly realised that considerable quantitative differences may occur within a given instar and between the different active stages. A survey of the literature shows that the susceptibility of insects to different contact insecticides has often been compared without any exact reference to the stage of development of the test subject. The stages used in toxicological estimation are more frequently adults, larvae or nymphs, less often eggs or pupae. With any given species, large variations in susceptibility may occur throughout the life-cycle and it would seem important for the purposes of practical control that some knowledge is available on

the change of resistance with the stage of development, both in order to assist the correct timing of the application and in order to assess the likelihood of resistant stages being present and their effect on the efficiency of practical control.

The main object of the present study of the problem of age-susceptibility relationship was to obtain data on the changes in resistance that occur as development proceeds. The resistance of each instar was examined, where possible, and the resistance of the different instars compared; where it was not possible to separate the instars with certainty, the nymphs or larvae were isolated in size groups and the resistance of each group determined and compared with one another. It was realised, however, that the resistance of any given instar is not constant throughout its duration; the resistance of an egg changes as development proceeds and these changes in resistance also occur in instars other than the eggs.

Data should be available on the changes in resistance that occur during instar development before a fully satisfactory comparison between instars can be made. Owing to lack of time and to technical difficulties, it was only possible to examine the change of resistance within an instar in certain selected instances, *i.e.*, the eggs and larvae of *Diataraxia oleracea* (L.), the pupae of *D. oleracea* and *Tenebrio molitor* L. and the adults of *T. molitor*. For the comparison of the difference in resistance that occurs between other instars, an arbitrary point in the development of each instar was selected and its resistance determined at that point. It is not possible, therefore, to state with certainty that the most resistant stage of development of one instar is compared with the most resistant stage of another. From the available information it would appear likely that the changes that occur between the instars are sufficiently large to render, in most instances, the differences that occur within the instar of secondary importance. For example, eggs of *D. oleracea* vary in their degree of resistance to DDT according to age and 12-hour-old eggs are found to be $1\frac{1}{2}$ times more resistant than the 120-hour-old eggs. But if the resistance to DDT between the instars such as egg and larva is compared, *e.g.*, 120-hour-old-eggs and 1st-instar larvae, it will show that the former are 3.35 times more resistant to DDT than the 1st-instar larvae. If comparisons are made between 12-hour-old eggs and 1st-instar larvae, the difference is even greater, being 5.5 times.

Review of the Literature.

A survey of the literature indicates that irrespective of the form in which the poison is administered, whether as contact insecticide, stomach poison or fumigant, the age at any stage of the life-cycle of a particular insect species appears to affect its resistance to the poison. The following is a short account of previous work.

Poison administered as fumigant.

Cotton (1932) working on the relation of the respiratory metabolism of insects to their susceptibility to fumigants showed that as there is a marked difference in the metabolic rate of the adults, larvae and pupae of *Tribolium confusum* Duv., and *Ephestia kühniella* Zell., they should show a corresponding difference in susceptibility to fumigants. He demonstrated that when test lots of 50 adults, 25 larvae and 25 pupae of *T. confusum* were placed in 6-litre glass flasks and fumigated for 3 hours at a temperature of 75°F. with varying quantities of carbon disulphide, the adults were killed with a dosage of 125 mg. per litre, larvae 163 mg. per litre and pupae 314 mg. per litre. He concluded that "other factors being equal, the susceptibility of an insect to a fumigant is influenced by any factor that affects the rate of metabolism of that insect. Any factor that increases the rate of metabolism increases the susceptibility of the insects to the action of a fumigant and vice versa".

Lindgren (1935) studied the order of resistance of different stages of *Tribolium confusum* to various fumigants and showed that with carbon disulphide the resistance

of the egg > pupa > adult > larva (low humidity), the pupa > egg > adult > larva (high humidity); with chloropicrin, the resistance of the egg > pupa > adult > larva; with ethylene oxide, the resistance of the pupa > adult > larva > egg. All the above data are based on tests on groups of individuals of various ages. In all cases, the effect was assessed on the basis of milligrams of fumigant per litre to give a 50 per cent. kill in five hours.

Gough (1939) investigating the relative resistance of different stages of *Tribolium confusum* to hydrogen cyanide found that 0 to 1-day-old eggs required 0.195 mg./litre hydrogen cyanide to give a 50 per cent. kill, whereas 3 to 4-day-old eggs required 0.326 mg./litre to give the same kill. The 20-day-old larvae required 0.439 mg./litre hydrogen cyanide to give a 50 per cent. kill; 0 to 1-day-old and 7 to 8-day-old pupae required 0.659 mg./litre and 0.729 mg./litre respectively, whereas 2 to 3-day-old pupae required 1.296 mg./litre to give a 50 per cent. kill. Thus, he found that recently formed pupae and those that were just about to emerge were much more susceptible than those in the middle period. In the case of adults, he found that as the age increased the resistance decreased. The 0 to 1-day-old adult required 0.810 mg./litre, 14-day-old adult 0.750 mg./litre and 28-day-old adult 0.703 mg./litre to give a 50 per cent. kill. The order of decreasing resistance was pupa > adult > larva > egg. These tests were all performed at 27°C. and 60 per cent. relative humidity and 1½ hours was the standard time of exposure.

Gunderson and Strand (1939) compared the toxicity of hydrogen cyanide, chloropicrin and ethylene oxide to eggs, nymphs and adults of the bed-bug, *Cimex lectularius* L. by exposing them to a number of different concentrations of each fumigant. The experiments were carried out at 25°C. in fumigation chambers. Two-day-old eggs, 2nd- and 3rd-instar nymphs and adults of various ages were used. The experimental insects were kept in contact with the different gases for 5 hours at 25°C. and were afterwards placed in glass vials at 27°C. The authors concluded that the eggs of the bed-bug are less resistant to hydrogen cyanide and ethylene oxide than the nymphs and adults. The eggs were apparently much more resistant to chloropicrin than the nymphs and adults.

Sun (1947) studied the effect of stage and age as a factor affecting the results of fumigation. He found that the susceptibility of *Tribolium confusum* eggs to carbon disulphide increased consistently with age, from 1 to 7 days. The susceptibility of larvae of various ages to CS₂ was different from that of the eggs; their susceptibility increased from 1 day to 7 days, and there was no appreciable change from 7 to 14 days. After that, their susceptibility decreased as the age increased. The pupal resistance to carbon disulphide differed greatly—according to whether the median lethal concentrations were compared on the basis of abnormal emergence or no emergence after fumigation. Adults from 1 day to 24 weeks old were fumigated with CS₂ and in general the older adults were more susceptible to the fumigant than young individuals but 7-day-old adults became more resistant than those 4 days old. It may be noted that the basis of assessment of toxicity in these experiments is the concentration of poison required to kill a given number of individuals.

Poison administered as stomach poison.

Campbell (1926) was the first to investigate age-susceptibility relations quantitatively in insects. He compared the susceptibility of the last four instars of silk-worm larvae to arsenic, on the basis of doses of poison per unit weight of insect which produced equal effects. The poison was administered orally by means of a micro-burette. The same volume of solution per gm. of larva (5 cu. mm.) was fed to each silkworm and it was found that the M.L.D. increased from about 0.015 to 0.025 mg. of arsenic per gm. of body weight during successive larval instars. He demonstrated that relative susceptibility, like relative toxicity, may be expressed numerically either as a ratio of the doses required to produce the same toxic effect (dosage-

comparison) or as a ratio of the effects produced by equal doses (effect-comparison). Displaying his data graphically and basing his comparison on dose in mg./gm. body weight he showed that 2nd-instar larvae were 3.5 times as susceptible to arsenic as 5th-instar larvae and concluded that the susceptibility of the silkworm to arsenic decreased during its larval development.

Contact poisons applied as a spray.

Newcomer and Yothers (1932) found in the laboratory that when highly refined oil was sprayed on codling moth eggs of different ages deposited on pear leaves or apples, ranging from less than a day old to 7 days (*i.e.*, those almost ready to hatch) a definite correlation existed between the age and percentage failing to hatch, the older the eggs the smaller the percentage that failed to hatch.

Hough and Jefferson (1936) found that there was no consistent relation between the age of the codling moth eggs and effectiveness of petroleum oil. In each test they used different but known ages usually ranging from less than one day to eggs due to hatch within a short time. On the other hand, with nicotine, the ovicidal efficiency increased as the age of the eggs increased. When nicotine sulphate (1 pint in 100 gallons) was used against eggs less than 47 hours old, which would not normally hatch until five and three-quarter days after the time of spraying, the kill was relatively low (67.1 per cent. hatched), but in the same test against eggs two and three days older, the kill was materially increased (0.2-0.6 per cent. hatched).

Simanton and Miller (1937) while working on house-fly age as a factor in susceptibility to pyrethrum sprays, found that very young flies were more easily paralysed but less easily killed than older flies. A spray containing 122 mg. of total pyrethrins in 100 ml. of deodorised petroleum oil was used. Tests were conducted by the Peet-Grady method and the following ages were used: 1-6 hours; 6 hours; 21 hours; 45 hours; 3 days; 4 days; 5 days; 8 days and 10 days old. They found that young adults were much more resistant than older flies. Woodbury (1938) worked on the relative susceptibility of the second instar, adult male and adult female of the German cockroach, *Blattella germanica* (L.). He found that the females were much more resistant to the action of the O.T.I. (official test insecticide—solution of pyrethrins in deodorised kerosene) than adult males of the same age. He confirmed this observation when he compared the quantity of insecticide deposited during the period of exposure necessary to kill 50 per cent. of the insects. The weight of the deposit per square centimetre required to kill 50 per cent. of the insects was 3.4 mg. per sq. cm. for the 2nd-instar nymph, 8.8 mg. per sq. cm. for adult males and 15.2 mg. per sq. cm. for adult females. He found that adult males were about $2\frac{1}{2}$ times and adult females $4\frac{1}{2}$ times as resistant to the official test insecticide as the second-instar nymphs. McGovran and Fales (1942) found that adult females were more resistant to pyrethrum-petroleum oil spray than adult males and large nymphs when treated with direct spray. The nymphs were intermediate in resistance between the adult males and females.

Tuma (1938), while studying the relationship between ages of *Blattella germanica* and their resistance to liquid insecticides, found that individuals 17-weeks old were the most resistant to the action of pyrethrins. He atomised 2 cubic centimetres of a pyrethrum spray in a "death chamber" which consisted of a standard 16-quart glass specimen jar, covered temporarily while the liquid insecticide was atomised, the volume being kept constant. The youngest nymphs 1-5 weeks old were less resistant than the older ones (*i.e.*, 20-25 weeks old), but the 17-week-old specimens were the most resistant. Bushland (1939) tested the ovicidal effectiveness of some common volatile (essential) oils on *Cochliomyia americana* Cush. & Patt. eggs. He divided the eggs into two classes, those less than 3 hours old and those within about 2 hours of hatching. Each oil was tested by dipping in it at least 10,000 eggs of each class and the younger eggs were found to be more easily destroyed than older eggs.

Ludwig (1946) studied the effect of DDT on the metabolism of the Japanese beetle, *Popillia japonica* Newm., using eggs, larvae, pupae and adults. For his studies on eggs he used groups of approximately 100 on successive days of embryonic development. The test subjects were exposed by placing them for a ten-minute period in a petri dish on the surface of a filter paper moistened with a 5 or 10 per cent. solution of DDT in peanut oil. The results showed that DDT had no effect on the embryonic development but it resulted in a slight retardation of hatching when the eggs were exposed early in development. He used the same technique when applying the poisons to the larval, pupal and adult stages. The 3rd-instar larvae were very susceptible. Pupae exposed to 5 to 10 per cent. DDT, in some cases had abnormal emergence and died subsequently. He found that DDT was toxic to adult beetles in concentrations as low as 0.2 per cent.

Du Chanois (1947) studied the toxicity of γ BHC to the pre-imaginal stages of the house-fly. The insects were placed on or between two discs of paper towelling cut to fit 50 ml. Stender dishes, to each of which was added a measured quantity of the insecticidal preparation. The age and stages used were eggs, 1-4 days old; 2nd-instar larvae, 2-3 days old; 3rd-instar larvae, 4-5 days old and pupae, 1-2 days old. He found that γ BHC was practically non-toxic to ova even at a high dosage level of 4.0-8.0 gm. per 100 ml., but a rapid post-incubation poisoning effect was exhibited. Second-instar larvae were the most susceptible, a dosage of 3.21 mg. per litre gave 50% mortality and the pupal stage was considerably more resistant than the larval stages.

Yosida (1948) worked on the toxicity of pyrethrins to certain insect larvae at different stages of growth using (I) *Bombyx mori* (L.), (II) *Barathra brassicae* (L.) and (III) *Chilo simplex* (Btlr.). The resistance of I at different stages was 1st < 5th < 4th > 3rd > 2nd instar, that of II, 3rd > 2nd > 1st and that of III, 4th < 3rd < 2nd < 1st instar assessed on the basis of the lethal concentration required to kill 50 per cent. and 99 per cent. of the treated individuals; when however the assessment was on the basis of the mean lethal concentration per unit body weight, this value was found to decrease with increasing insect age. Smith and Pearce (1948) who studied the mode of action of petroleum oils as ovicides, found that when the eggs of *Cydia* (*Grapholitha*) *molesta* (Busck) at different stages of development (8, 30, 54 and 78 hours old) were treated with sub-lethal dosages of oil by means of a settling mist technique, the resulting hatch indicated a difference in susceptibility during the first two-thirds of their incubation period. Increasing resistance to the oil was noticed in the last third of the incubation period, and they concluded that as the age of the eggs increased, the resistance increased. Chapman and Pearce (1949) compared oil sprays, prepared from a single product, for their ovicidal efficiency against winter eggs of the European red mite. They found that when it was applied at the beginning of the hatching period and at the 6th, 16th, 21st and 39th day preceding this event, susceptibility of the eggs increased as the intervals between spraying and the time of hatching were reduced.

Discussion of previous work.

The work described above provides conclusive evidence that the susceptibility of an insect to any given poison varies with its stage of development, where the criterion of toxicity is the concentration of poison required to kill a given number of individuals. These changes in susceptibility occur when poisons are applied as fumigants, stomach poisons or contact poisons.

Some evidence is put forward to show that when poisons are applied as fumigants, differences in susceptibility are correlated with differences in metabolic rate, but it is doubtful whether this is more than a partial explanation, since it does not take account of the qualitative morphological, physiological and biochemical changes that occur during development.

Only one series of experiments has been carried out with a stomach poison (Campbell, 1926) and then it was found that not only did the larval resistance increase throughout larval development when judged on the amount of poison required to kill a given number of individuals, but also when judged on the amount required to kill a given weight of living material, on the basis of mg. of poison per kg. body weight. No explanation of these changes in resistance has been put forward.

The criterion of toxicity used almost exclusively in the published work on the action of contact poisons is that of the concentration required to kill a given number of individuals. Any study of the effect of contact poisons is complicated by the technical difficulty of assessing the dosage, *i.e.*, the amount of poison retained by the insects and in this respect there is a great, perhaps fundamental, difference between poisons applied in this way and those applied as fumigants or stomach poisons. This matter is discussed in the body of the paper.

Technique.

Method of rearing the test insects.

The tomato moth, *Diataraxia oleracea* (L.), the American cockroach, *Periplaneta americana* (L.), and the mealworm, *Tenebrio molitor* L., were the three species of test insects used. The details of rearing under approximately standard conditions are given below.

Diataraxia oleracea (L.).

This species which belongs to the Agrotidae, a family of considerable economic importance, has proved to be a satisfactory plant-feeding insect for laboratory rearing. It has the advantage of prolific egg production, ease of rearing and resistance to disease of the larvae, and a wide choice of food plants one or other of which is available throughout the year. Full details of the method employed for rearing this insect are given by Way, Smith and Hopkins (1951). The particular techniques employed for obtaining individuals of known age are outlined below.

Breeding cages comprising a glass battery jar 1 ft. 2 ins. in height, 10 ins. in length, 8 ins. in breadth covered with muslin were used for the eggs (Way, Smith and Hopkins, 1951). Twenty or more adults were kept in the cages and fresh sugar solution was provided every other day. Every morning the batches of eggs laid on the muslin cover of the cage were cut out with the muslin to which they were attached and kept in closed glass dishes in the C.T. room at 25°C. and 60 per cent. R.H. labelled with the date of laying. The age of these eggs was recorded as 12-hours old because it was noticed that moths usually lay their eggs between 6 p.m. and 8 p.m. in the evening, thus eggs of known age ranging from 12-hours old to 144-hours old were obtained.

Caging is necessary at all stages of larval development, particularly during the early instars when they stray very readily from the food plant. The leaves of potted dock plants were enclosed in fine muslin bags (Way, Smith & Hopkins, 1951). Egg batches which hatched within a known period were placed on the leaves and the bag tied at either end, these pots were labelled with the date of hatching of the larvae. They were kept in the C.T. room at 25°C. and 60 per cent. R.H. under artificial lighting conditions. Fresh foliage was provided by severing the old leaf from the plant and inverting the bag containing it over a new leaf. Batches of larvae of the first three instars were obtained in this way. When the larvae reached the 3rd instar they were transferred to a large cage measuring 24 ins. in length, 14 ins. in height, 12 ins. in width (Way, Smith & Hopkins, 1951), having a wooden framework, glass top and muslin sides, which fitted closely on to a 2½ ins. deep pupation box. Dry peat was placed in the box to a depth of 2 ins., jam jars containing cut leaves of dock, brussels sprouts or cabbage were stood on a tray and crumpled brown paper was

placed between the jars. Later stages, *i.e.*, 4th- and 5th-instar larvae were selected from these cages.

As far as possible larvae of the same age were bred in each cage ; this is essential if all are to pupate at the same time. After each day's collection, the pupae were placed in 13 cm. crystallising dishes and kept at a temperature of 25°C. and 60 per cent. R.H., each dish being labelled with the date of pupation. By collecting over a period, successive batches of pupae of known age were obtained.

It was necessary to prevent diapause which occurs in the pupal stage ; accordingly larvae at the later stages (4th- and 5th-instar) were reared in cages as described above under artificial light provided by a 60-watt tungsten or 80-watt tubular fluorescent lamp, continuously for sixteen hours per day (Way, Hopkins & Smith, 1949).

Periplaneta americana (L.).

In order to obtain a continuous supply of cockroaches of known age, two types of cages were used. The first was a single large stock-cage for development of collections and general maintenance and the second a large number of small cages in which insects from a single day's hatching from egg capsules, kept in petri dishes, were reared until ready for use.

The stock cage consisted of a metal tank 4½ ft. long, 2 ft. high, 2 ft. wide. The top was divided into two parts, the first of glass framed in wood and the other made of wood, the two sections being attached together by clips ; running around the rim of the cage were two brass strips insulated from the tank and connected to a dry battery of 120 volts, forming an electric fence to keep the insects from escaping while collecting oöthecae or feeding. A constant temperature of 30°C. inside the cage was maintained by two heater mats thermostatically controlled. The bottom of the cage was covered with 1½ ins. layer of coarse sawdust. Pieces of pleated paper and flower pots placed in piles served as hiding places for adults and large nymphs, while strips of corrugated cardboard were employed as shelters for small nymphs (see Pl. III). Fresh food was provided as necessary, and consisted of rolled oats and potatoes, and an automatic water supply was provided by inverting a jar of water in a dish with cotton wool. The food was placed in petri dishes located near the shelters.

Every day 26–29 egg capsules (oöthecae) were collected from the stock cage. Only those egg capsules which were deposited in the sawdust were collected and care was taken to ensure that no oöthecae were left behind. After collection, the oöthecae were kept in petri dishes labelled with the date of deposition and put in the constant temperature cabinet at 30°C., until they hatched. By collecting over a period, batches of oöthecae of different ages were obtained.

The petri dishes were examined daily and newly hatched nymphs were transferred to a 13 cm. crystallising dish labelled with the date of hatching ; in this way a continuous chain of breeding cages containing nymphs of known age was maintained. These crystallising dish cages for nymphs were provided with rolled oats and water, and covered at the top with muslin held in place by a rubber band. Cones of filter paper about 2–3 ins. tall and 1½ ins. in diameter at the base were used in these cages as a retreat for the young nymphs. By this means six distinct age and size groups of nymphs were isolated.

Due to technical difficulties and the long duration of the adult life of *P. americana*, which is between 5–6 months, it was only possible to provide two age groups, young adults and old adults.

Tenebrio molitor L.

The beetles were bred on wheat bran and potatoes in iron bins in a constant temperature room at 25°C. and 60 per cent. R.H. The following technique was employed to obtain the various stages at a known age.

Mature male and female beetles were kept in bins packed with bran to a depth of six inches. After 48 hours these adults were removed from the bins and the bins numbered and dated. On the third day after removal of the adult males and females, the bin was examined and the bran sieved through a $\frac{1}{16}$ -in. mesh sieve on a metal tray and young larvae of the 1st size were obtained.

As moulting could not be ascertained definitely, several cultures were started in the bins and larvae selected from them according to their size and weight. Six size-groups were used for the larvae of this species.

A number of last instar (6th size) larvae in the prepupal stage were collected from the larval bin and kept in a separate bin, labelled and dated. As soon as they pupated they were collected in 13 cm. crystallising dishes with the dates of pupation on them and kept at 25°C. and 60 per cent. R.H. By this means successive batches of pupae of varying ages from 1 day old to 8 days old were obtained.

The 8-day-old pupae, about to emerge into adults, were collected from the pupal bins and kept in a separate bin labelled "adults". The emerging adults were collected daily from these bins and kept in crystallising dishes labelled with the date of emergence, giving successive batches of adults of known ages.

The average weight of all the batches of insects was determined before treatment.

Insecticides used.

The following insecticides were used :—

- (i) DDT.—The pure 2,2 bis (parachlorophenyl) 1,1,1 trichloroethane. A DDT suspension of different concentrations, containing needles 50 μ long was prepared as described by McIntosh (1947) and used in an aqueous medium containing 1 per cent. w/v saponin, 10 per cent. v/v alcohol.
- (ii) Pyrethrins.—A stock solution of 1.03 per cent. w/v total pyrethrins in acetone was made from a commercial extract containing 25 per cent. w/v total pyrethrins and kept in the dark in a refrigerator at 2°C. and used in an aqueous medium containing 1 per cent. w/v saponin and a varying percentage of acetone up to 5 per cent. v/v.

The spray deposits over the whole series of experiments ranged from 6.13 to 9.17 mg./sq. cm., but 223 out of the 268 recorded deposits lay between 7 and 9 mg./sq. cm.

The environmental conditions of spraying were uncontrolled; the humidity ranged from 33 per cent. to 70 per cent., and the temperature from 14°C. to 27°C. throughout the whole series, but the great majority of the experiments were carried out within a range of 50–65 per cent. R.H. and 18–25°C.

The apparatus used throughout was that described by Potter (1952).

Details of treatment.

Although the details of treatment varied slightly according to the test insect and the insecticide used, the general technique employed was standardised, and is described here to avoid repetition.

No particular deviation was made from Tattersfield and Potter's (1943) method of direct spraying. The general technique involved three major operations (a) direct spraying in petri dishes (b) maintenance of insects in the petri dish under standard conditions of temperature (c) assessment of effect.

Direct spraying.

Tattersfield and Potter's (1943) method of direct spraying was employed throughout the experiments. The insects were treated in a spraying apparatus described by Potter (1952), which is designed to give an even deposit over the sprayed area and

provide a wide range of reproducible deposits. The test insects were confined in a petri dish of 9 cm. diameter containing a Whatman No. 1 filter paper and then sprayed directly with 5 cc. of liquid insecticide. Various concentrations of the liquid insecticide were used, but the volume of the liquid and the area of the sprayed surface were kept constant. After the spraying operation was completed, each petri dish containing treated insects was covered with a muslin top held in place by a rubber band and then transferred to a constant temperature room or cabinet either at 25°C. or 20°C. Active feeding stages were supplied with food one hour after treatment when the spray film had dried. The food was placed on a coverslip to avoid contact with the treated petri dish and thus prevent stomach poisoning incidental to feeding.

Maintenance of insects after treatment.

After spraying, the insects were kept in the petri dish until the assessment of effect was completed. The insects were provided with fresh food daily.

A point which needs clarification is the change of "after-treatment" temperature from 25°C. as employed for DDT to 20°C. for the treatments with pyrethrins. In preliminary tests carried out to find the range of concentrations of pyrethrins to kill larvae of *Diataraxia oleracea* it was found that the larvae were very resistant when kept at 25°C. after treatment. A concentration of 2 per cent. pyrethrins was required to give 100 per cent. kill of the 5th-instar larvae of *D. oleracea* and with the material available there was blockage of the atomising nozzle of the spray machine. As it was thought that even higher concentrations might be required for other stages, as subsequently proved to be the case, some means was sought of increasing the toxicity of the pyrethrins.

It was considered possible that the relatively low toxicity of the pyrethrins at 25°C. might be due to the high after-treatment temperature. Potter and Gillham (1946) studied the effect of temperature before and after spraying on the toxicity of various contact poisons to adult *Tribolium castaneum* (Hbst.) with pyrethrin and other insecticides. They tested the effect of temperature after spraying and in all cases they found higher toxicity at lower temperatures. Harries and others (1945) working on some factors affecting the insecticidal action of pyrethrum extracts on the beet leaf-hopper, *Eutettix tenellus* (Baker), found higher toxicity with higher temperature during spraying and with lower temperatures after spraying.

In view of the evidence given above and the data obtained from the preliminary tests, an experiment was carried out to determine the relative toxicity of pyrethrins on the different instars of *D. oleracea* larvae at two different "after-treatment" temperatures, 20°C. and 25°C. respectively. The results are given in Table I.

TABLE I.

Effect of two different "after-treatment" temperatures on the toxicity of pyrethrins to *D. oleracea* larvae—5th instar.

Concentration gm./100 cc.	Percentage mortality at 25°C. (after treatment)	Percentage mortality at 20°C. (after treatment)
2.0 per cent.	100	100
1.0 " "	86	100
0.5 " "	73	96
0.25 " "	67	88

The above figures show that at "after treatment" temperatures of 20°C., the relative toxicity of pyrethrins increased as compared with "after treatment" temperatures of 25°C. On the basis of the findings of previous workers and the data obtained from the above experiments it was decided to change the "after treatment" temperature for pyrethrins from 25°C. to 20°C.

Assessment of effect.

Many methods of determining the mortality of the insects after spraying have been suggested in the literature. The standard method of assessing the toxicity of insecticides as described by Tattersfield and Potter (1943) was followed, except in a few cases discussed more fully later, where other classifications were used. The insects were examined individually and in the case of adults and larvae they were assigned to one of the following five categories : (1) normal (N), those in which no sign of abnormality existed after spraying and the insects were feeding ; (2) slightly affected (S), those suffering some inhibition of movement but feeding ; (3) badly affected (B), those with definite sign of paralysis and powers of movement greatly restricted—not feeding ; (4) moribund (M), those which appear to be dead but when disturbed show feeble movements of some part of the body ; (5) dead (D), those which show no sign of life despite probing with a needle.

The following classification was employed for eggs : (A) eggs dead in the process of embryonic development ; sometimes the embryo formed normally inside the egg but the larva did not hatch ; (B) eggs developed normally but the larvae died at emergence, *i.e.*, embryonic development was completed in the normal period as evidenced by the appearance of the larval jaws and outline of the body, but the larvae died while breaking through the chorion ; (C) dead—no embryonic development of the egg ; (D) hatched normally.

The following categories were used in respect of the pupae : (1) normal emergence, when the pupal development was complete and the adult emerged in the normal period ; (2) incomplete emergence when the adult was killed towards the end of metamorphosis and the abdomen remained enclosed in the pupal case ; (3) no emergence when death occurred in the process of pupal development.

The results of the toxicity tests with DDT and the pyrethrins were analysed by the standard probit technique. Although the method had been carefully standardised, small day-to-day variations, both in the median lethal concentration and in the slope of the probit line, frequently occurred. Some of these variations may be attributed to difference in the insect cultures employed : but, in addition, there were unexplained variations. The differences observed between different age groups were, however, quite constant.

The validity of the assumption that the dosage proportional to the body weight of each individual should be taken as a criterion for assessing the toxicological response to certain poisons, could be questioned because in many instances, and especially where the toxic agent is a contact poison, susceptibility is more likely to be proportional to the surface of absorptive tissue than to the mass (or volume) of the entire body. It has been previously pointed out that when a contact poison is applied directly to an insect it is difficult to estimate how much of the poison is deposited on the surface of the body, how much is absorbed by the tissues concerned, and how much reaches the site of action to give the required kill.

In field applications of insecticides for the control of pests, it is convenient to know at what percentage concentration the poison is effective. Such a procedure has been adopted by previous workers as shown in the review of the literature. With this in mind all the data in the following experiments were based on the median lethal concentration required to kill 50 per cent. of the population of individuals at a given stage of development and the test subject was said to have increased in resistance or *vice versa* if the concentration required to kill 50 per cent. of the individuals of the population increased or decreased irrespective of the size or weight of the individuals comprising that population. A consideration of the relationship between concentration-mortality, surface area and body weight is given in the discussion.

RELATIVE RESISTANCE OF DIFFERENT STAGES OF THE TEST INSECTS TO DDT AND THE PYRETHRINS.

Eggs of Diataraxia oleracea.

It was found that the eggs of *D. oleracea* could easily be separated out individually after soaking in water and the small pieces of muslin with the egg batches attached were cut out and put in water for a few seconds to soak. After soaking they were put on dry filter paper and the eggs were separated out individually with a fine brush soaked in water.

Four distinct age groups (12, 36, 60 and 120 hours old) of eggs were isolated for this test.

These eggs of different ages were treated by means of a direct spray. The total number of eggs used for each experiment was 420. Each replica contained from 10 to 20 per dish. The number of replicates was three.

The first inspection was made as soon as the eggs in the control hatched.

To DDT.

DDT had no effect on embryonic development ; this is in accord with the observations of Ludwig (1946) who used eggs of *Popillia japonica*. Embryonic development was completed in the normal period as evidenced by the appearance of the larval jaws and outline of the body which are visible through the chorion. Ludwig (1946) noted a slight retardation with eggs of *P. japonica*. A rapid post-incubation poisoning was evidenced by the fact that as soon as the larva tried to gnaw its way out of the egg chorion it collapsed inside the egg itself (Pl. III *a* and *b*) and, where this occurred, the eggs were recorded as killed. Only where the larvae emerged completely were the eggs recorded as having survived the treatment, and these larvae were rapidly killed by contact with the residual film of DDT on the substratum.

The results based on the post-incubation poisoning effect are shown in Table IV and Graphs 1 to 3. This post-incubation poisoning effect was not observed to occur until the larva, which had developed normally inside the chorion, started to gnaw the egg shell in order to emerge and, therefore, probably death was due to stomach poison effect in all instances.

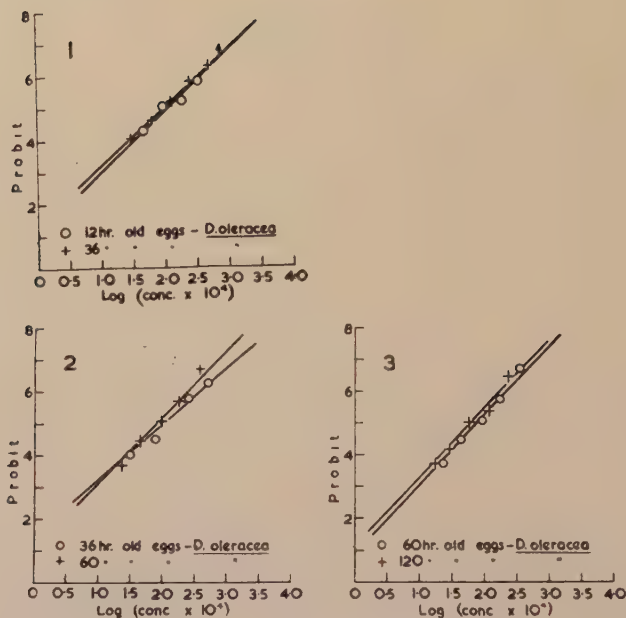
TABLE II.

Effect of differences of age of eggs of *D. oleracea* on their resistance to DDT.

Age in hours	Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration	Relative resistance
12	2.0441 ± 0.15	1.81 ± 0.25	0.011	1.6
36	2.0054 ± 0.07	1.84 ± 0.30	0.010	1.3
60	1.9270 ± 0.13	2.33 ± 0.26	0.0084	1.1
120	1.8254 ± 0.14	2.11 ± 0.25	0.0067	1.0

Table II shows that the resistance of the eggs to DDT decreased consistently as the development proceeded. The 12-hour-old eggs were found to be approximately $1\frac{1}{2}$ times as resistant as the 120-hour-old eggs.

The increase in susceptibility with age appears to proceed at a uniform rate from 12 hours to 120 hours.



Graphs 1-3.—Probit mortality—log concentration graphs showing the effect of difference of age of *D. oleracea* eggs on their susceptibility to DDT.

Probit mortality—log concentration regression equations :—

$$12\text{-hour-old eggs } Y = 1.8146x + 1.30.$$

$$36\text{-hour-old eggs } Y = 1.8416x + 1.31.$$

$$60\text{-hour-old eggs } Y = 2.3310x + 0.51.$$

$$120\text{-hour-old eggs } Y = 2.1155x + 1.13.$$

To pyrethrins.

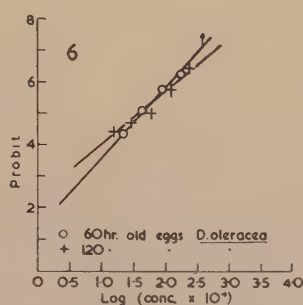
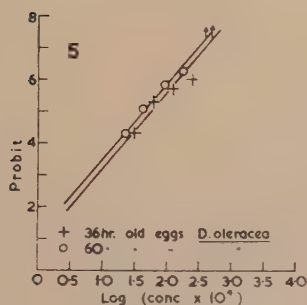
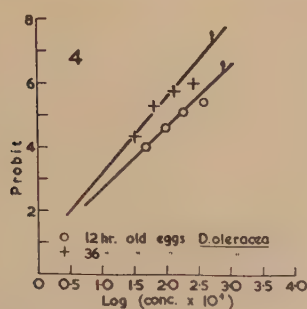
The effect of pyrethrins on the different ages of eggs of *D. oleracea* was studied on the same age groups as those used for the experiments with DDT. The technique and the details of the treatment were essentially similar, except that the after-treatment temperature was 20°C. The reason for this change has already been explained above (p. 129).

The first inspection was made soon after the eggs in the control hatched.

During each observation the eggs were classified into four categories A, B, C, and D, as previously defined (p. 130). The pyrethrins were ovicidal and had an effect on the embryonic development of some of the eggs. Some affected eggs developed as usual and darkening of the egg shell occurred, but the larvae did not hatch after the normal incubation period. The eggs in which the embryo did not develop became darker in colour and the chorion became opaque. Those eggs which failed to hatch and also those in which the embryo did not develop were both considered dead and were assigned to category "A". Those which hatched, even though they eventually died due to the film effect of the pyrethrins, were considered normal.

Three experiments were made with each age group, giving a total of twelve experiments for the four age groups. The total number of eggs used in each experiment varied from 270 to 600, the total number in the twelve experiments being 5,570. Three replicates were used at each concentration.

The results are shown in Table III and Graphs 4 to 6.



Graphs 4-6.—Probit mortality—log concentration of poison showing the effect of difference of age of *D. oleracea* eggs on their susceptibility to the pyrethrins.

Probit mortality—log concentration regression equations :—

$$12\text{-hour-old eggs } Y = 2.0342x + 0.59.$$

$$36\text{-hour-old eggs } Y = 2.2724x + 1.00.$$

$$60\text{-hour-old eggs } Y = 2.2435x + 1.32.$$

$$120\text{-hour-old eggs } Y = 1.5922x + 2.38.$$

TABLE III.

Effect of differences of age of eggs of *D. oleracea* on their resistance to pyrethrins.

Age in hours	Log median lethal concentration x 10 ⁴	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
12	2.1724 ± 0.06	2.03 ± 0.31	0.015	3.5
36	1.7621 ± 0.12	2.27 ± 0.10	0.0057	1.3
60	1.6428 ± 0.13	2.24 ± 0.25	0.0044	1.02
120	1.6478 ± 0.06	1.59 ± 0.24	0.0043	1.0

The results set out in Table III show that the resistance of the eggs of *D. oleracea* to pyrethrins consistently decreases as development proceeds. The eggs were kept at 25°C. until they were treated and the incubation period at this temperature is approximately 144 hours so that embryonic development was far advanced in the 120-hour old eggs. The increase in susceptibility with age did not appear to proceed at a uniform rate, from 12-36 hours there was a marked increase in susceptibility followed by a levelling off during the 36-120-hour period, during which susceptibility only increased slightly.

Hough & Jefferson (1936) found that with nicotine the ovicidal efficiency increased as the age of the eggs increased, for example when nicotine sulphate was used against eggs less than 47 hours old, which did not normally hatch until five and three-quarter days after treatment, the kill was relatively low (67.1 per cent. hatched), but in the same test against eggs two and three days later, the kill was materially increased (0.2-6 per cent. hatched). For various insect eggs, Fink (1925), Melvin (1928), Bodine (1929) and Burkholder (1934), have shown that there is an early "formative period" and during this early embryonic development the metabolic activity is comparatively low. This "formative period" is followed by a period of high metabolic activity. It may be that the increase in susceptibility of the older eggs to pyrethrins which is shown in Table III is because the insecticide is not as effective during the "formative period", but when the embryo is developed the increased metabolic activity results in the toxic action being exerted and the embryo is killed.

Larval instars of D. oleracea.

A larval culture of *D. oleracea* was maintained to make it possible to select considerable numbers of larvae of any desired instar or age for the toxicity tests. The age of the larvae is indicated in terms of instars of which there are five. Five age groups were isolated and the larvae of any given instar were not used until two days after moulting. In addition to the age, the individual weight of the larvae at each particular age or instar was obtained. The experiments and the details of the treatment were essentially similar to those of preceding experiments.

A source of variation in toxicological estimation lies in the selection of samples to be used in the tests. In these experiments, samples were obtained by drawing a few insects from the culture, or from a mixture of several cultures, with a small brush, the process being repeated until a sample of sufficient size was obtained. A few hours before spraying, the larvae were removed from the mass sample and transferred to clean specimen tubes in the numbers required for the separate replicate. The number of larvae used per petri dish for each test was five.

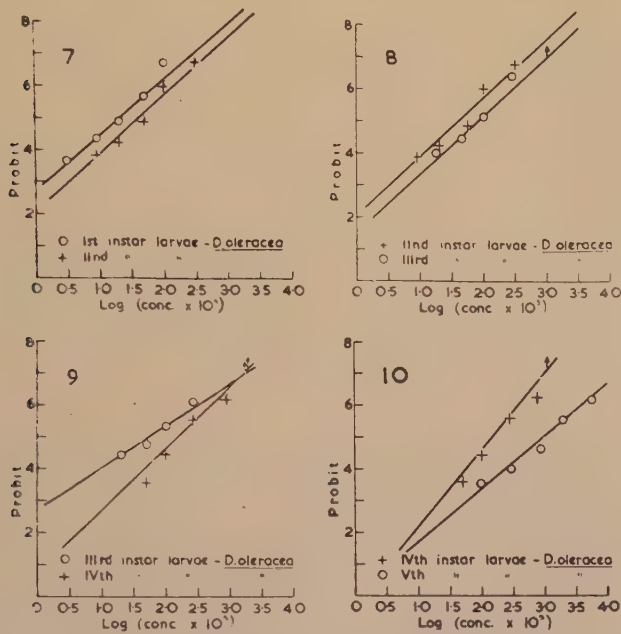
To DDT.

In order to determine the concentration-mortality regression line, three sprayings were carried out with each instar making a total of 15 experiments with five instars. For most of the experiments a total of 175 larvae were used. Five replicates, each of five insects, were used at each concentration. A total of 2,545 insects were used in the 15 experiments. Food consisting of cabbage leaves was given to the larvae after the filter paper substratum in the dish was dry.

The first inspection of the dishes was made after 24 hours. During the inspection the larvae were classified into five categories, normal, slightly affected, badly affected, moribund and dead, but in the final analysis all insects not dead were counted as normal.

Soon after exposure to DDT the affected individuals became very active and eliminated a considerable quantity of faecal matter or sometimes regurgitated; they exhibited the violent muscular tremors which accompany DDT poisoning.

The results were analysed and are set out in Table IV, the concentration mortality regression lines are shown in Graphs 7-10. A significant deviation from parallelism occurred with the comparison between the 3rd- and 4th-instar larvae; with the other instars the toxicity could be compared at any level.



Graphs 7-10.—Probit mortality—log concentration of poison showing the effect of difference in larval instars of *D. oleracea* on their susceptibility to DDT.

Probit mortality—log concentration regression equations :—

1st-instar larvae $Y=1.8076x+2.67.$
2nd-instar larvae $Y=1.9156x+1.95.$
3rd-instar larvae $Y=2.1866x+0.89.$
4th-instar larvae $Y=2.2363x+0.11.$
5th-instar larvae $Y=1.6405x+0.05.$

TABLE IV.
Effect of difference in larval instar of *D. oleracea* on their resistance to DDT.

Instar	Weight (mg.)			Log median lethal concentration x 10 ⁴	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative Resistance
	Max.	Min.	Av.				
1st	0.27	0.17	0.20	1.2839±0.07	1.81±0.10	0.002	1
2nd	3.5	2.5	3.0	1.5969±0.07	1.91±0.31	0.004	2
3rd	15.0	9.0	12.0	1.8940±0.11	2.18±0.31	0.006	3.1
4th	132	100	116	2.1830±0.07	2.24±0.30	0.016	8
5th	580	500	510	3.0180±0.09	1.64±0.26	0.10	50

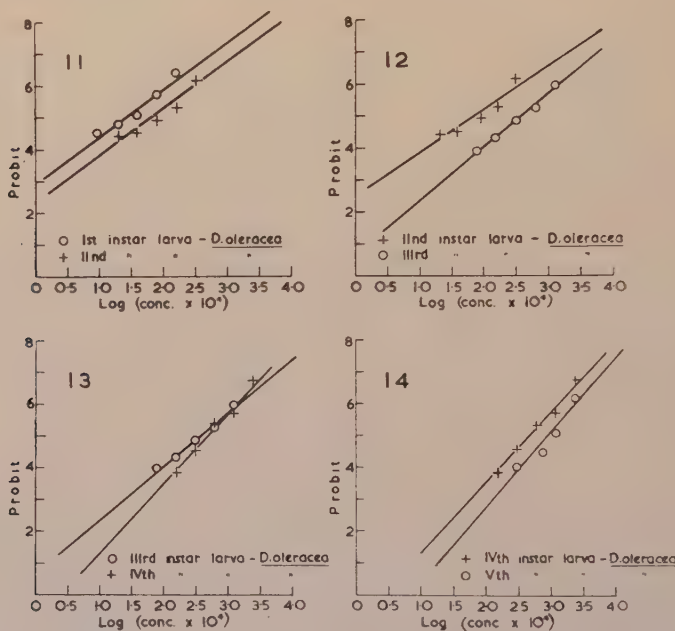
From Table IV it is apparent that higher concentrations are required to kill older larvae than younger. This trend is the reverse of that obtained with eggs where the older eggs were more susceptible than the younger.

To pyrethrins.

With the pyrethrins two sprayings were made with each instar to determine the position of the regression line so that with the five instars ten experiments were carried out in all. Five replicates of five insects were used at each concentration and a total of approximately 175 insects for each experiment. The total number of insects used was 1,249. An hour after treatment the insects were provided with cabbage leaves.

The first inspection of the dishes was made 24 hours after spraying. Observations were repeated daily until no further mortality was recorded.

Soon after exposure to pyrethrins the larvae became very active and eliminated a considerable amount of faecal matter and in some cases the posterior portion of the alimentary canal was found to be thrust out of the anus. The 1st-instar larvae at lower concentrations did not eat much of the food provided but later instars ate more freely, the difference in the amount eaten probably being correlated with difference in paralytic rate.



Graphs 11-14.—Probit mortality—log concentration of poison showing the effect of difference in larval instars of *D. oleracea* on their susceptibility to the pyrethrins.

Probit mortality—log concentration regression equations :—

$$\begin{aligned} \text{1st-instar larvae} & Y=1.471x+2.93. \\ \text{2nd-instar larvae} & Y=1.3602x+2.48. \\ \text{3rd-instar larvae} & Y=1.6436x+0.75. \\ \text{4th-instar larvae} & Y=2.2486x-1.09. \\ \text{5th-instar larvae} & Y=2.4041x-2.21. \end{aligned}$$

The probit regression lines calculated from the data are shown in Graphs 11-14. There is no significant deviation from parallelism between any of the lines, except in the case of the 3rd and the 4th instar, where the lines tend to converge at the apex.

Table V shows an analysis of the data obtained.

TABLE V.

Effect of differences in larval instar of *D. oleracea* on their resistance to pyrethrins.

Instar	Weight (mg.)			Log median lethal concentration 10^4	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
	Max.	Min.	Av.				
1st	0.27	0.17	0.21	1.4040 ± 0.09	1.47 ± 0.30	0.0027	1
2nd	4.0	3.0	3.3	1.8542 ± 0.09	1.36 ± 0.30	0.0081	3
3rd	21.0	10.0	15.0	2.5828 ± 0.07	1.64 ± 0.31	0.041	15
4th	132	100	116	2.7103 ± 0.06	2.25 ± 0.33	0.054	20
5th	580	500	540	3.0009 ± 0.06	2.40 ± 0.37	0.10	37

Table V. shows that the changes in susceptibility to pyrethrins throughout the larval life of *D. oleracea* were different from that which occurs during the development of the egg. Each successive instar was more resistant to the insecticide.

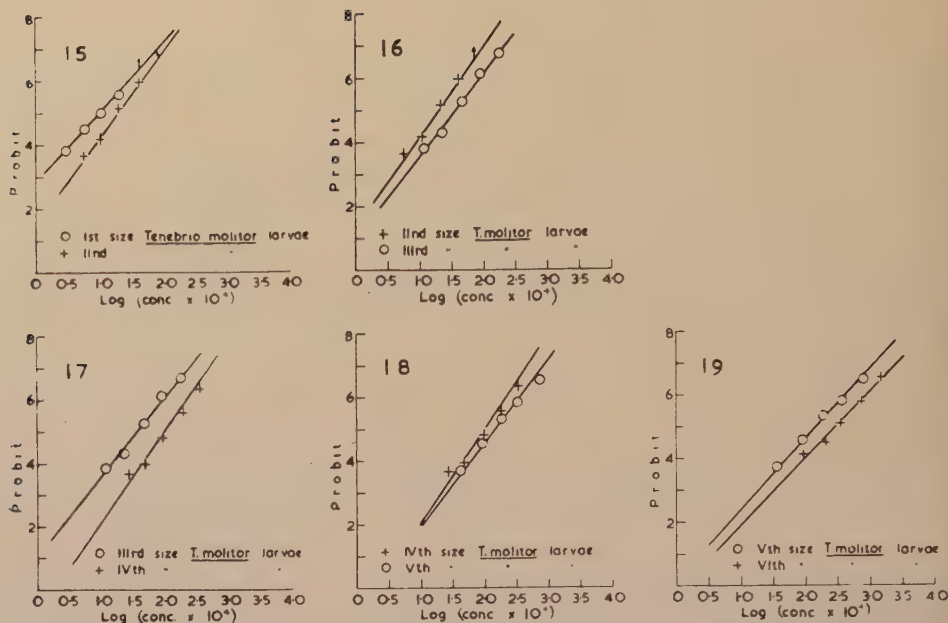
Groups of larvae of Tenebrio molitor.

A large culture of *T. molitor* was maintained for this study. As the different instars could not be ascertained, larvae were graded according to size, *i.e.*, 1st–6th size (Pl. IV). In weight, each size was approximately double that of the previous size and, to be more accurate, in every experiment the test larvae were weighed before being sprayed. In this way an approximate average weight for each size was also obtained. An hour before treatment, larvae of the required size were sieved out and kept in crystallising dishes; the number used in each test varied from 5 to 10 per petri dish. The technique was the same as in all the previous experiments. An hour after spraying food was provided in the form of pieces of potato placed on glass coverslips to avoid any stomach poison effects and the food was changed every 24 hours.

To DDT.

With this poison two tests were made of the susceptibility of each size group making 12 tests in all. For the first three size groups approximately 175 insects were used and for the remaining three groups approximately 300 insects in each test, so that approximately 2,850 insects were used to cover all six size groups.

The first inspection was made 24 hours after spraying and it was noticed that the larvae remained apparently normal in all the concentrations, the effect of poisoning being gradual. Accordingly it was decided to inspect every alternate day. Throughout the test, poisoning was for the most part progressive and on every alternate day larvae classified in one category had to be removed to the next category, *e.g.*, normal—slightly affected—badly affected—moribund—dead. At lower concentrations, however, larvae that were classified as slightly affected in the earlier inspections sometimes recovered and were later found to be normal. The poisoning symptoms were the same as those shown by *D. oleracea* larvae; there were violent muscular tremors and twitching and wriggling of the body. In the final analysis all the insects not dead were counted as normal. Observations were repeated for a period of 14 days, *i.e.*, until all concentrations showed no further kill.



Graphs 15-19.—Probit mortality—log concentration of poison showing the effect of difference in larval size group of *Tenebrio molitor*, on their susceptibility to DDT.

Probit mortality—log concentration regression equations :—

1st size larvae	$Y = 2.3203x + 2.69.$
2nd size larvae	$Y = 2.8800x + 1.21.$
3rd size larvae	$Y = 2.6110x + 0.88.$
4th size larvae	$Y = 2.3958x + 0.12.$
5th size larvae	$Y = 2.2527x + 0.07.$
6th size larvae	$Y = 2.0114x - 0.01.$

Some details of the probit analysis of the data are given in Table VI together with the M.L.C.'s and the relative resistances of the different size groups. Graphs 15-19 show the probit regression lines. No significant deviation from parallelism between any of the lines was noticed.

TABLE VI
Effect of difference in larval size of *T. molitor* on their resistance to DDT.

Size group	Weight (mg.)			Log median lethal concentration 10^4	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
	Max.	Min.	Av.				
1	7.0	3.0	5.0	1.0996 ± 0.06	2.32 ± 0.30	0.00125	1
2	14.0	10.0	12.0	1.3159 ± 0.05	2.88 ± 0.26	0.002	1.6
3	33.0	21.0	27.0	1.5785 ± 0.05	2.61 ± 0.28	0.0038	3.0
4	46.0	40.0	43.0	2.0418 ± 0.05	2.39 ± 0.39	0.011	8.8
5	63.0	60.0	61.0	2.1911 ± 0.05	2.25 ± 0.39	0.015	12.0
6	128	110	119	2.4826 ± 0.05	2.01 ± 0.35	0.03	14.0

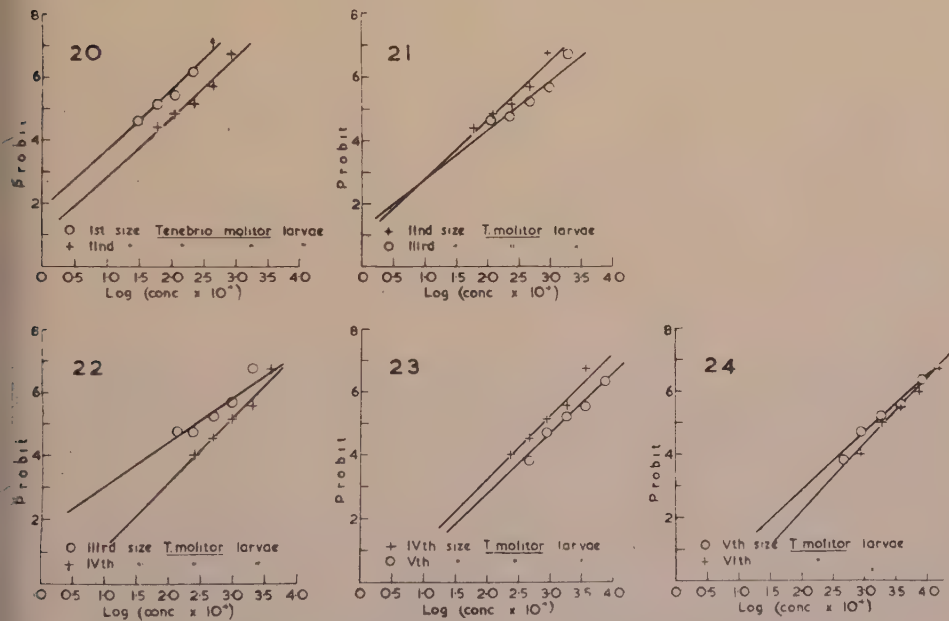
The results set out in Table VI show that the resistance of different size groups of larvae of *T. molitor* to DDT consistently increased as the age increased.

To pyrethrins.

The procedure followed was the same as that adopted for DDT. Two tests were made of the susceptibility of each size group. Approximately 175 insects were used in each test throughout the series so that approximately 2,100 were used in all.

The first inspection was made after 24 hours and it was noted that the larvae in all the treatments showed signs of poisoning. They were motionless and appeared to be moribund but if probed with a needle or disturbed with a brush, the larvae rolled over and over. It is perhaps interesting to note that a proportion of the larvae that appeared moribund in the earlier inspections were found later to have fully recovered. Recovery, however, only occurred at lower concentrations. The full effect of the poison could be assessed within 14 days; after this no change in mortality figures occurred. Earlier signs of poisoning consisted of unco-ordinated and violent movements of the legs. The criteria used for the assessment of the effect were the same as before.

The data are set out in Table VII and Graphs 20-24.



Graphs 20-24.—Probit mortality—log concentration poison showing the effect of difference in larval size groups of *Tenebrio molitor* on their susceptibility to the pyrethrins.

Probit mortality—log concentration regression equations :—

- 1st size larvae $Y = 1.8425x + 1.85.$
- 2nd size larvae $Y = 1.6904x + 1.32.$
- 3rd size larvae $Y = 1.4219x + 1.57.$
- 4th size larvae $Y = 2.0418x - 0.90.$
- 5th size larvae $Y = 1.893x - 1.03.$
- 6th size larvae $Y = 2.09x - 1.96.$

TABLE VII.

Effect of differences in larval size of *T. molitor* on their resistance to pyrethrins.

Size group	Weight (mg.)			Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
	Max.	Min.	Av.				
1	5.6	4.0	4.8	1.7072 ± 0.06	1.84 ± 0.32	0.005	1
2	20.0	10.0	15.0	2.1743 ± 0.07	1.69 ± 0.32	0.013	2.6
3	35.0	23.0	29.0	2.4111 ± 0.06	1.42 ± 0.35	0.027	5.0
4	45.0	31.0	38.0	2.8911 ± 0.10	2.04 ± 0.34	0.072	14.2
5	68.0	50.0	59.0	3.1846 ± 0.08	1.89 ± 0.34	0.14	28.0
6	140	100	120	3.3278 ± 0.08	2.09 ± 0.36	0.2	40

Table VII shows that, as with DDT, susceptibility of various sizes of larvae of *Tenebrio molitor* to pyrethrins decreased with increasing size.

Groups of nymphs of Periplaneta americana.

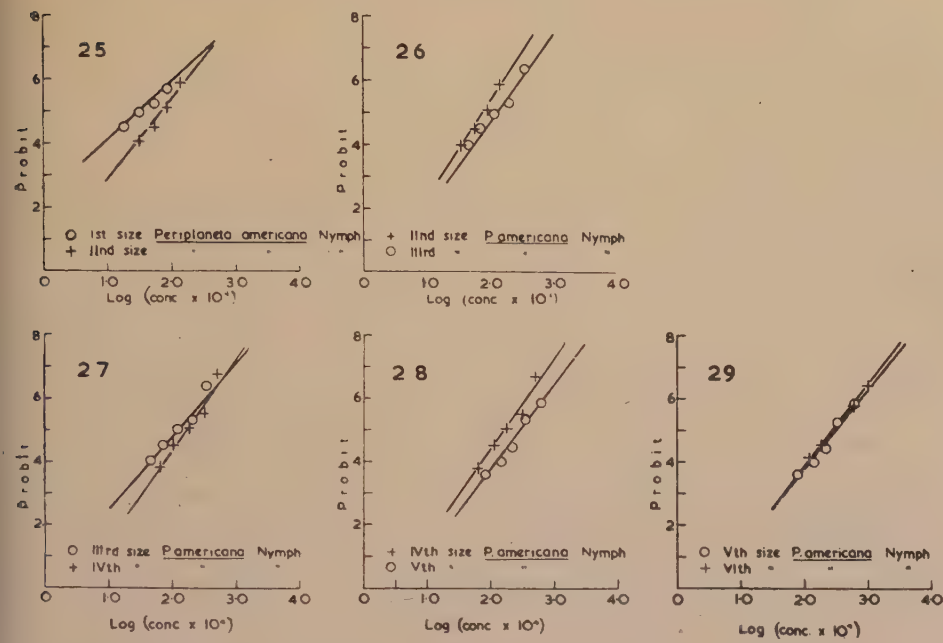
A large culture of *P. americana* of all stages was maintained to make it possible to select considerable numbers of individuals of any desired age or size for the test. In this species, too, it was found to be impossible to distinguish between the instars and nymphs were therefore divided into size groups. The weight of a sample of individuals in each size group was determined and the average weight of an individual of each size was estimated. Accordingly, throughout the test the age is indicated by size group and weight. The individuals of each group were approximately double the size of the members of the preceding group (Pl. IV). The size groups varied from 1 week to 12 weeks in age; they were reared in different cages according to their sizes and samples were drawn from them as necessary. An hour before treatment the nymphs were removed from their culture and inactivated by chilling in a refrigerator at 5°C. The number of nymphs used for each petri dish varied from 2 to 10. With the smaller size nymphs, the usual number of individuals per petri dish was 10; as the size increased the number per petri dish fell. Food consisted of pieces of potato placed on coverslips.

To DDT.

Two tests were carried out with each size group, so that twelve tests were carried out in all. The numbers for each test ranged between 100 and 300, and the total number of insects used was approximately 2,300. Five replicates were used at each concentration. The application procedure was the same as in the preceding experiments.

The first inspection was made 24 hours after spraying. Inspections were carried out on alternate days and continued for ten days until no increase in mortality was recorded in any of the treatments. The typical symptoms of DDT poisoning were observed, as described by Tobias and others (1946). These authors described the sequence of symptoms in *P. americana* as (1) hyperextension of legs, elevation of centre of gravity, postural instability, (2) increasing general tremulousness involving head, body and appendages, (3) ataxic gait and hyperactivity resulting from stimuli of sound and touch, (4) animal repeatedly falling on its back and finally unable to rise, (5) leg movements continuing with two components, a high frequency tremor, and (see p. 147) a slower flexion and extension, (6) fast tremors disappearing, leaving only isolated motions of body wall, tarsi, palpi, cerci, and antennae and (7) the final sign of life being the beating of the heart which may continue for a day or more. Most of the above symptoms were noticed in the bigger sizes, e.g., 3rd, 4th, 5th and 6th sizes of nymphs of *P. americana*. Tobias and his collaborators consider these symptoms to be typical of neuromuscular poisoning.

The results are shown in Table VIII and Graphs 25-29.



Graphs 25-29.—Probit mortality—log concentration of poison showing the effect of difference in nymphal size groups of *Periplaneta americana* on their susceptibility of DDT.

Probit mortality—log concentration regression equations :—

- 1st size nymphs $Y=2.1559x+1.67.$
- 2nd size nymphs $Y=3.1073x-0.84.$
- 3rd size nymphs $Y=2.4314x-0.06.$
- 4th size nymphs $Y=2.7687x-1.19.$
- 5th size nymphs $Y=2.6067x-1.43.$
- 6th size nymphs $Y=2.4307x-0.99.$

TABLE VIII.

Effect of difference in nymphal size of *P. americana* on its resistance to DDT.

Size group	Weight (mg.)			Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
	Max.	Min.	Av.				
1	5.8	5.4	5.2	1.5416	2.15 ± 0.40	0.0035	1
2	11.0	9.0	10.0	1.8718	3.11 ± 0.31	0.0074	2.1
3	41.9	31.0	36.0	2.0823	2.43 ± 0.43	0.012	3.4
4	87.0	75.0	87.0	2.2346	2.77 ± 0.45	0.017	4.8
5	190	110	150	2.4636	2.61 ± 0.45	0.027	8.2
6	290	210	250	2.4650	2.43 ± 0.60	0.03	8.5

Table VIII shows that the susceptibility to DDT of various sizes of nymphs of *P. americana* decreased consistently as the age increased.

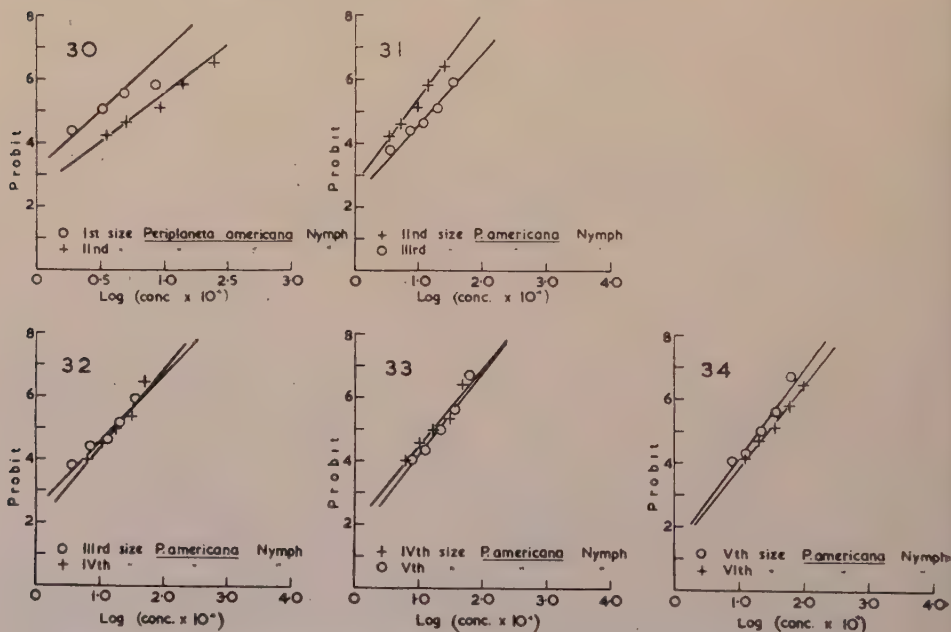
To pyrethrins.

The effect of pyrethrins was tested on nymphs of different ages of *P. americana* grouped according to sizes as described in the DDT tests. The experiments and details of the treatments were essentially similar to those of the preceding experiments. The number of nymphs per petri dish varied from 2 to 10. Two tests were made with each size group, the number per test ranging from 100 to approximately 800 and the total

number of insects used was approximately 2,200. Five replicates were used for each concentration. An hour before treatment the insects were removed from their culture and transferred to clean crystallising dishes which were put into a refrigerator for an hour at 5°C. thus rendering the nymphs inactive.

The first inspection was made 24 hours after spraying and subsequently inspections were carried out on alternate days over a period of 8 days. The poison appeared to have exerted its maximum effect by the 8th day since there was no increase in mortality between the 6th and 8th days. The general toxic effect of pyrethrins on cockroaches has been studied by Hutzel (1942) who measured the activation rate on *Blattella germanica* by an entomographic method. After a short latent period averaging 2 seconds with oil solutions and 5.5 seconds with dusts, there followed a period of intense excitement during which the running rate of the insect increased from 3 to 11 cm. per second. The second phase of poisoning was submaximal activity in which the leg muscles showed signs of incomplete relaxation. In the series of experiments described in this paper, some poisoning effect was noticed in nymphs of all age groups—especially the larger sizes, *i.e.*, 3rd, 4th, 5th and 6th sizes. After 24 hours, complete knock-down had occurred in all the concentrations. Some of the paralysed insects recovered and others died, the percentage dying being dependent on the concentration used. Pyrethrins, therefore, exercise their maximum paralytic effect within 24 hours and paralysis is reversible since insects with low doses recover. They thus differ from DDT which causes gradual paralysis over a period of days, from which the insects seldom, if ever, recover.

The results are shown in Table IX and Graphs 30–34.



Graphs 30–34.—Probit mortality—log concentration of poison showing the effect of difference in nymphal size groups of *Periplaneta americana* on their susceptibility to the pyrethrins.

Probit mortality—log concentration regression equations:—

- 1st size nymphs $Y = 2.2422x + 3.91.$
- 2nd size nymphs $Y = 2.7159x + 2.70.$
- 3rd size nymphs $Y = 2.2636x + 2.55.$
- 4th size nymphs $Y = 2.4028x + 0.02.$
- 5th size nymphs $Y = 2.7859x + 1.33.$
- 6th size nymphs $Y = 2.4529x + 1.43.$

TABLE IX.

Effect of difference in nymphal size of *P. americana* on its resistance to pyrethrins.

Size group	Weight (mg.)			Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
	Max.	Min.	Av.				
1	5.8	4.0	4.9	0.4866 ± 0.06	2.24 ± 0.43	0.00031	1
2	13.0	11.0	12.0	0.8487 ± 0.03	2.72 ± 0.32	0.00071	2.3
3	42.0	30.0	36.0	1.1903 ± 0.06	2.26 ± 0.43	0.0015	4.9
4	84.0	78.0	81.0	1.2417 ± 0.05	2.40 ± 0.43	0.0017	5.5
5	190	130	160	1.3201 ± 0.05	2.78 ± 0.43	0.0021	6.7
6	310	210	260	1.4571 ± 0.07	2.45 ± 0.56	0.003	10.0

Table IX shows that the resistance of various sizes of nymphs of *P. americana* to pyrethrins increased consistently with age.

Pupae of Tenebrio molitor.

In order to select a considerable number of individual pupae of *T. molitor* of different ages, a number of prepupae were collected from the larval bin, labelled and dated. As soon as they pupated they were collected in 13 cm. crystallising dishes with dates of pupation on them and kept at 25°C. and 60 per cent. R.H. Successive batches of 1-, 4- and 8-day-old pupae were obtained in this way. In these tests the age is indicated in terms of days. Under the conditions of the experiment the normal pupal period is 8-9 days.

To DDT.

The method of treatment was the same as that in the previous experiments the number of pupae used being five per petri dish.

Two tests were made with each age group making six tests in all. Approximately 105 pupae were used in the first two tests and approximately 175 pupae for each of the four subsequent tests; thus approximately 910 pupae were used in the whole experiment.

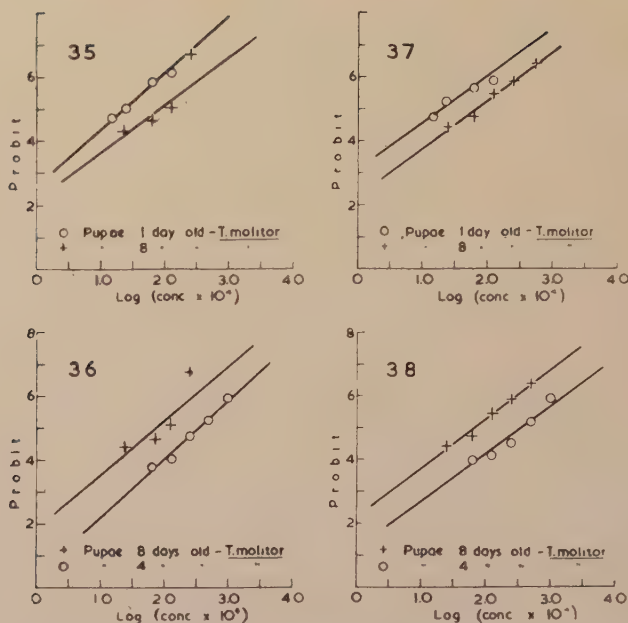
The first inspection was made on the 9th day after spraying and inspection was continued until all the pupae in all the concentrations either emerged normally or died.

The insects were divided into three classes according to the effect of the poison: (1) normal emergence in which the pupal development was completed and the adult emerged in the normal period, (2) incomplete emergence in which the adult was killed during metamorphosis and the abdomen remained enclosed in the pupal case, (3) no emergence in which death occurred in the process of pupal development.

Although the pupae were separated into the above categories during inspection, in the final analysis only those insects that emerged normally were counted as being alive, the rest were regarded as having been killed. In the exploratory experiments it was found that even with concentrations of DDT as high as 0.5 per cent. pupal development was not prevented although the fully formed adult was killed prior to emergence.

The pupal sheaths surrounding the abdomen and elytra, which normally rupture just prior to emergence, remain intact in the poisoned pupae. It seems possible that the DDT interferes with the metabolism of the pupa so that the adult when it is formed is weakened and unable to emerge or that the metabolism of the pupal sheath itself is deranged and it does not split in the normal manner.

The results are shown in Table X and Graphs 35 and 36.



Probit mortality—log concentration of poison showing the effect of difference in pupal ages of *T. molitor* on their susceptibility to DDT and the pyrethrins.

Graphs 35–36.—DDT.—Probit mortality—log concentration regression equations :—

1-day-old pupae $Y = 1.6794x + 2.72$.

4-day-old pupae $Y = 1.8412x + 0.31$.

8-day-old pupae $Y = 1.6847x + 1.84$.

Graphs 37–38.—Pyrethrins.—Probit mortality—log concentration regression equations :—

1-day-old pupae $Y = 1.4203x + 3.13$.

4-day-old pupae $Y = 1.4642x + 1.28$.

8-day-old pupae $Y = 1.5727x + 2.10$.

TABLE X.

Effect of differences in pupal age of *T. molitor* on its resistance to DDT.

Age in days	Log median lethal concentration x 10 ⁴	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1	1.3571 ± 0.12	1.68 ± 0.12	0.0023	1
4	2.5489 ± 0.07	1.84 ± 0.31	0.035	15
8	1.8809 ± 0.08	1.68 ± 0.27	0.0075	3.2

Table X shows that the recently formed pupae (1-day old) and those just about to emerge (8-days old) were much less resistant than those in the middle period, i.e., 4 days old.

To pyrethrins.

The susceptibility of different ages of *T. molitor* pupae to pyrethrins was tested in the same manner as that just described for DDT, and the numbers used in each test followed the same pattern.

The first inspection was made on the 9th day after spraying and the measurement of the toxic effect was based on the classification described in the preceding experiment. As with DDT, the pyrethrins, even at the high concentrations of 2.5 per cent. did not prevent the development of the adult within the pupa, but caused an incomplete emergence followed by death. Incomplete emergence seemed to follow the same course as that previously described for DDT although it is difficult to imagine that the mechanism of toxic action in the two cases is the same.

The analysis of the results is set out in Table XI and Graphs 37 and 38.

TABLE XI.

Effect of differences in age of the pupae of *T. molitor* on their resistance to pyrethrins.

Age in days	Log median lethal concentration 10^4	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1	1.3169 ± 0.05	1.42 ± 0.12	0.0021	1
4	2.5479 ± 0.08	1.46 ± 0.30	0.035	16.6
8	1.8471 ± 0.05	1.57 ± 0.29	0.007	3.3

Table XI shows that the recently formed pupae (1-day old) and those just about to emerge (8-days old) were much less resistant than those in the middle period (4-days old).

Pupae of *D. oleracea*.

Pupae of 1-, 10- and 20-days old were used and, since under the conditions of the experiment the pupal period is about 21 days, the insects within these age groups may be considered to be young, middle aged and old respectively.

To DDT.

DDT was tested on all the pupal age groups and found to have no toxic effect with concentrations up to 0.5 per cent. w/v. Since this is the highest concentration that could be used in practice, it was not possible to determine the susceptibility of this stage of *D. oleracea* to DDT.

To pyrethrins.

The method of application was the same as that used in the previous experiments. The number of pupae used per petri dish varied from 2-6 in different tests. Three replicates were used at each concentration and two tests were made in each age group. The pupae of this insect were difficult to obtain in large numbers, and the number for each test varied from about 84 to 144. The total number used in the experiment was approximately 630.

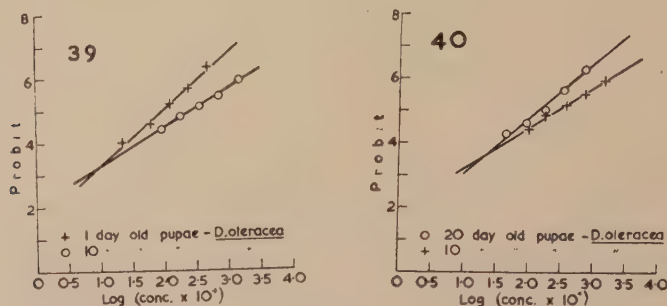
The first inspection was carried out on the 21st day after spraying in all cases. Inspection continued until all the pupae were either dead or emergence had taken place. The same method of classifying the effect of treatment was used with the pupae of this species as had been used with the pupae of *T. molitor*.

The data were finally analysed on the basis that only insects that emerged normally were counted as alive and the rest dead. This was necessary because in exploratory experiments it was found that a complete kill of the pupae, *i.e.*, prevention of partial emergence, could not be achieved even at a concentration of 2.5 per cent. w/v of the pyrethrins.

Insects were classified under the heading of incomplete emergence, when adult development had been completed. It was found that in every case this had occurred even when there was no rupture of the pupal case, in fact there was no evidence that

pyrethrins prevented the development of the adult within the pupa even at the highest concentrations that were applied.

An analysis of the results is given in Table XII and Graphs 39 and 40.



Graphs 39-40.—Probit mortality—log concentration of poison showing the effect of difference in pupal ages of *D. oleracea* on their susceptibility to the pyrethrins.

Probit mortality—log concentration regression equations :—

$$\text{1-day-old pupae } Y = 1.7446x + 1.54.$$

$$\text{10-day-old pupae } Y = 1.1875x + 2.10.$$

$$\text{20-day-old pupae } Y = 1.6737x + 1.38.$$

TABLE XII.

Effect of differences in the age of pupae of *D. oleracea* on their resistance to pyrethrins.

Age in days	Log median lethal concentration x 10 ⁴	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1	1.9885 ± 0.10	1.74 ± 0.43	0.0097	1
10	2.4445 ± 0.12	1.19 ± 0.33	0.028	2.9
20	2.1677 ± 0.11	1.67 ± 0.45	0.015	2.3

Table XII shows that the recently formed pupae (1-day old) and those just about to emerge (20-days old) were less resistant than those of the middle period (10-days old).

Adults of Tenebrio molitor.

The insects for this series of experiments were produced by the method described on the section on technique, adults of 1, 4 and 8 weeks old being used.

The total adult life of *Tenebrio molitor* at 30°C. is 8 to 9 weeks and the age groups selected could, therefore, be called young, middle-aged and old adults. Throughout this test "age" is indicated in terms of "weeks".

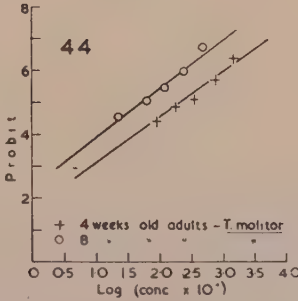
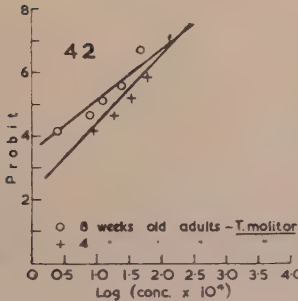
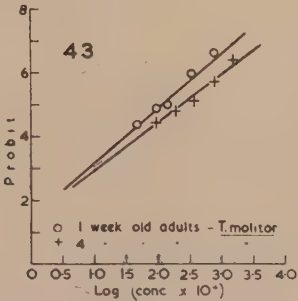
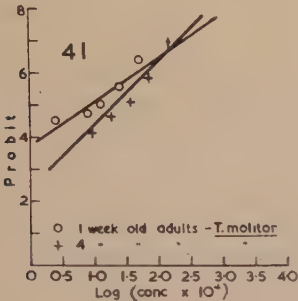
To DDT.

The application technique was as before. The number of individuals per petri dish varied from 5 to 10, five replications being used at each concentration. Two tests were carried out on each age group and the number of individuals used for each test varied from approximately 105 to approximately 175, the total number for the whole experiment being approximately 900. Pieces of potato placed on coverslips were provided as food for the insects an hour after spraying.

The first inspection was made 24 hours after spraying and inspections were continued until no increase in kill was recorded in two subsequent inspections. The total duration of the period of examination was 12 days.

It was noticed that soon after exposure to DDT spraying the adults became very active and eliminated a considerable amount of faecal material. The symptoms shown by these insects, as poisoning progressed, followed closely those described by Tobias and others (1946) for *Periplaneta americana* namely ataxic gait and hyperactivity, when the insect repeatedly fell on its back and finally was unable to regain its feet. Leg movements continued with two components, a high frequency tremor and a slower flexion and extension.

The results are shown in Table XIII and Graphs 41 and 42.



Probit mortality—log concentration of poison showing the effect of difference in adult ages of *T. molitor* on their susceptibility to DDT and the pyrethrins.
Graphs 41–42. DDT.—Probit mortality—log concentration regression equations :—
1-week-old adults $Y=1.3750x+3.76$.
4-week-old adults $Y=3.8969x-0.70$.
8-week-old adults $Y=1.7018x+3.33$.
Graphs 43–44.—Pyrethrins.—Probit mortality—log concentration regression equations :—
1-week-old adults $Y=1.6285x+1.62$.
4-week-old adults $Y=1.4824x+1.54$.
8-week-old adults $Y=1.5565x+2.29$.

TABLE XIII.
Effect of difference in age of adult *T. molitor* on their resistance to DDT.

Age group weeks	Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1	0.9012 ± 0.09	1.37 ± 0.12	0.0008	1
4	1.4600 ± 0.03	3.90 ± 0.30	0.0029	3.6
8	0.9082 ± 0.08	1.70 ± 0.30	0.0008	1

Table XIII shows that 4-week-old (middle-aged) adults were the most resistant to DDT.

To pyrethrins.

The same general technique was used as in the preceding experiments. The number of insects for these tests were 5 per petri dish and two tests were carried out with each age group. The number of individuals used in each test varied from approximately 105 to approximately 350; the total number of individuals used for the whole experiment was approximately 2,400.

The first inspection was made after 24 hours and was repeated on alternate days until no increase in mortality was observed in two consecutive inspections.

The progress of poisoning was found to be lack of co-ordination of hind legs, all legs affected but insects still able to walk, and, finally, the insect fell on its back and remained almost motionless until death. The knock-down was complete within 24 hours in all the concentrations, but it was found that in some of the lower concentrations the insects revived and were later classified as normal.

The results are shown in Table XIV and Graphs 43 and 44.

TABLE XIV.

Effect of differences in the age of adult *T. molitor* on their resistance to pyrethrins.

Age group weeks	Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1	2.0736 ± 0.10	1.63 ± 0.12	0.012	2.2
4	2.3378 ± 0.09	1.48 ± 0.29	0.022	4.1
8	1.7372 ± 0.07	1.56 ± 0.29	0.0054	1

Table XIV shows that with pyrethrins the old (8 weeks) adults were the least resistant, the middle-aged (4 weeks) adults the most resistant and the young (1 week) adults intermediate in resistance.

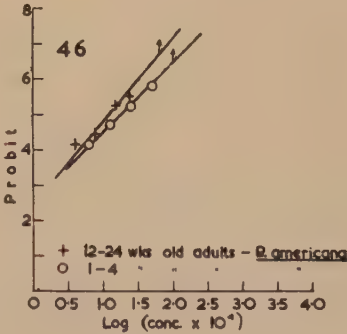
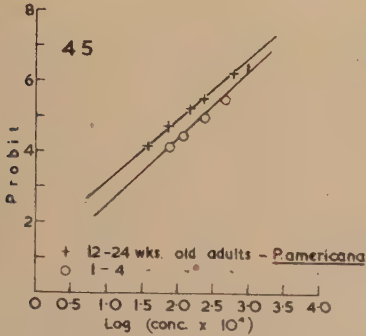
Adults of Periplaneta americana.

Under the conditions of the experiment, adults of this species generally live for six months or longer. Two age groups, 1-4 weeks old (young adults) and 12-24 weeks old (old adults) were isolated. These two age groups were maintained in separate cages. The ages chosen were entirely arbitrary and they were selected only from the point of view of convenience in rearing. Two tests were carried out with each age group and 70 insects were used for each test, so that approximately 280 individuals were used in all.

To DDT.

The general technique was the same as in the preceding experiments. The petri dishes after being sprayed were covered with zinc mesh tops which were held in place with rubber bands. Food, consisting of pieces of potatoes on coverslips, was given to the insects after the filter paper in the dish was dry. The first inspection was made 24 hours after spraying and was repeated on alternate days until no increase in mortality was recorded in two subsequent inspections. The symptoms of poisoning appeared shortly after spraying and the poisoning followed the course already described for the nymphs of this species.

The results are shown in Table XV and Graph 45.



Probit mortality—log concentration of poison showing the effect of difference in adult ages of *Periplaneta americana* to DDT and the pyrethrins.

Graph 45.—DDT.—Probit mortality—log concentration regression equations :—
Young, 1-4 weeks old adults $Y=1.9824x+0.38$.
Old, 12-24 weeks old adults $Y=1.7368x+1.43$.

Graph 46.—Pyrethrins.—Probit mortality—log concentration regression equations :—
Young, 1-4 weeks old adults $Y=2.1428x+2.36$.
Old, 12-24 weeks old adults $Y=2.7921x+2.00$.

TABLE XV.

Effect of difference in age of adult *P. americana* on their resistance to DDT.

Age group weeks	Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1-4	2.3333 ± 0.10	1.98 ± 0.48	0.021	1.9
12-24	2.0517 ± 0.11	1.74 ± 0.52	0.011	1.0

Table XV shows that the young adults are 1.9 times as resistant as the old adults to DDT.

To pyrethrins.

The same age groups were used as with DDT and the same general technique applied. Two tests of each age group were carried out and 70 insects were used for each test so that 280 insects were used in all.

After spraying, the petri dishes were covered with zinc mesh tops which were held in place with rubber bands. Food, consisting of cut pieces of potatoes on a cover slip, was provided after the filter paper was dry.

The first inspection was made 24 hours after treatment. Inspections were continued on alternate days until two consecutive inspections showed no change in the mortality. Symptoms of poisoning were noticed shortly after spraying and were the same as those already described for the nymphs. The recovery and survival of a percentage of insects that were initially paralysed also occurred as in the case of the nymphs.

The results are shown in Table XVI and Graph 46.

TABLE XVI.
Effect of difference in age of adult *P. americana* on their resistance to pyrethrins.

Age group weeks	Log median lethal concentration $\times 10^4$	Slope of the probit line (b)	Median lethal concentration per cent. w/v	Relative resistance
1-4	1.2336 ± 0.11	1.74 ± 0.52	0.0017	1.4
12-24	1.0753 ± 0.07	2.79 ± 0.54	0.0012	1.0

Table XVI shows that young adults are 1.4 times more resistant to pyrethrins than the old adults.

Discussion.

Assessment of toxicity and comparison of the effect of DDT and the pyrethrins.

In the introduction it was pointed out that the main object of the present work was to study the changes in susceptibility that occur in the different developmental stages of an insect species. Two difficulties at once arise, the first of which is the estimation of the significance of any comparisons that are made.

Where the estimate is based on the concentration of poison, required to kill a given number of individuals, it is clear from the work described here that considerable variation of resistance may occur during the growth of an instar as well as between instars. Thus, the middle-aged pupa of *T. molitor* is 16.6 times as resistant to pyrethrins as the young pupa, and the middle-aged adult is 4.4 times as resistant as the young adult. If the resistance of the pupa is compared with that of the adult, the ratio obtained will be dependent on the age at which the test of susceptibility of each instar is made, consequently, any comparison will be of limited significance and will be liable to be misleading if the extent of variation within the instar is not taken into account. This has not always been done by previous workers.

It has not been possible to carry out detailed studies of the changes in resistance that occur during any instar, but some information was obtained on the variation occurring within the egg, pupal and adult stages of the species under investigation. The larval and nymphal stages, however, presented some difficulty. It was not

TABLE XVII.
Effect of difference in larval age within an instar of *D. oleracea* on resistance to pyrethrins.

Stage	Age	Median lethal concentration total pyrethrins	Relative resistance
1st instar 	4 hours	0.0005 per cent.	1
	24 hours	0.0018 per cent.	3.6
	54 hours	0.0025 per cent.	5.0
	72 hours	0.001 per cent.	2.0
2nd instar 	4 hours	0.0021 per cent.	1
	24 hours	0.006 per cent.	2.9
	48 hours	0.008 per cent.	3.9
	72 hours	0.0042 per cent.	2.0
3rd instar 	4 hours	0.007 per cent.	1
	24 hours	0.031 per cent.	4.4
	52 hours	0.037 per cent.	5.2
	72 hours	0.028 per cent.	4.0
	96 hours	0.018 per cent.	2.5

possible to distinguish the separate instars of the nymphs of *P. americana* or the larvae of *T. molitor*, so the figures obtained for the resistance of the different age groups had to be accepted as representative. Some further experiments were carried out with the 1st-, 2nd- and 3rd-instar larvae of *D. oleracea* using pyrethrins to determine the variation occurring within the larval instars of this species. The results of this test are set out in Table XVII.

This Table shows that in all three instars the larvae four hours after moulting were markedly less resistant than they were for the remainder of the instar, resistance then rises until at least the middle of the instar followed by a decline towards the end. All the tests described in this paper were carried out on larvae 24 hours after moulting and, judging from this evidence, the figures obtained will approximate to the maximum resistance of the instar.

In the following pages where comparisons have been made between instars, the period of maximum resistance has been chosen as the basis since this was thought to have the greatest practical significance.

The second difficulty was the choice of the basis on which to assess toxicity. As has already been pointed out, toxicity may be assessed either on the concentration of poison applied at a constant deposit level which will kill a given percentage of individuals, or on the amount of poison required to kill a given weight of insect material. With the technique employed, the relative toxicity on the first basis will be very different from that on the second if there are differences in size and shape between the groups that are being compared. The basis used here has been the concentration of poison required to kill a given percentage of individuals, irrespective of their size, when the spray is applied evenly to give a fixed constant amount per unit area. Where a comparison is being made of the resistance within an instar which does not change in size, such as the egg, pupa or adult, it seems reasonable to assume that the figures obtained for changes in resistance on the basis of the concentration applied to kill a given percentage of individuals also represent the changes in resistance on the basis of the amount of poison required to kill a given weight of insect material. Where, however, changes both in size and shape are involved such as comparisons between the egg and the larva, it is only possible, with the data available, to make comparisons on the basis of the concentration of poison required to kill a given percentage of individuals. It seems therefore that in general, while changes of resistance within an instar can be assessed on both bases, changes of resistance between instars can only be assessed on one. A further complication that is left out of account here is that active stages will pick up more poison than is deposited on them at the time of spraying by crawling about on the residual film of insecticide. It is not possible to assess this effect although it is recognised as important.

By making a number of assumptions, the ratio of the amount of poison to kill an insect of given weight may be calculated from the weight w of the insect and the concentration c of the poison applied. Assuming that the amount of spray retained by a unit area of insect is constant K_r , the amount of poison retained P_r is given by $P_r = K_r (\text{surface area}) c$, where c is the concentration of poison in the spray.

If it is also assumed that the ratios of the linear dimensions of the insect are irrespective of the size of the insect then

$$P_r = K_A L^2 c \quad (1)$$

where K_A is a constant relating the surface area of the insect with L a linear measure of the size of the insect.

Making the further assumption that the density of the insect is a constant d , then the weight of the insect w can be expressed as

$$w = K_v L^3 d = K_w L^3 \quad (2)$$

where K_v is a constant relating the volume of the insect with L . and $K_w = K_v \cdot d$. Hence the ratio of the weight of poison retained to the weight of the insect is

$$P_{r/w} = \frac{K \cdot K_A L^2 c}{K_w L^3} \quad (3)$$

from equation (2) we may substitute for L in terms of weight in equation (3) hence—

$$P_{r/w} = \frac{K \cdot K_A \cdot c}{K_w} \cdot \frac{1}{\sqrt[3]{w/K_w}}$$

Taking logs.

$$\log P_{r/w} = \log \frac{K \cdot K_A}{K_w} + \frac{1}{3} \log K + \log c - \frac{1}{3} \log w = \text{const} + \log c - \frac{1}{3} \log w$$

i.e. $\log (\text{poison per unit body weight}) = \log c - \frac{1}{3} \log w + \text{const.}$

By substituting in this formula the M.L.C.'s and the insect weights that have been obtained experimentally, the relative toxicity of the poisons to the various larval and nymphal instars on the basis of the amount of poison required to kill 50 per cent. of the total weight of insect material has been worked out, and is shown in Table XVIII. The Table shows that while there has been an increase in resistance with increased larval development judged on this basis, it is much smaller than on the other basis of the concentration of poison required to kill a given number of individuals.

It must be emphasised that in all the subsequent discussion the basis for comparison is the concentration required to kill a given percentage of individuals.

Table XVIII shows how resistance to DDT and pyrethrins changes with development within a species and between species.

The following comparisons may be made of the resistance between instars. With *D. oleracea* the most resistant stage to DDT is the pupa, which was not killed by concentrations up to 0.5 per cent. w/v the highest that it was possible to apply. This is followed in order of decreasing resistance, by the 5th- and 4th-instar larvae, the 12-hours-old eggs and finally the 3rd-, 2nd- and 1st-instar larvae. With pyrethrins the most resistant stage is the 5th-stage larva followed by the 4th and 3rd, the middle-aged pupa, the 12-hour eggs and the 2nd-instar larva. With the exception of the pupa which is resistant to DDT, the concentrations required to kill were of the same order with both poisons, the variation being from 0.002 per cent. (1st-instar larva) to 0.10 per cent. (5th-instar larva) for DDT, a difference of 50 times and 0.0027 per cent. (1st-instar larva)—0.10 per cent. (5th-instar larva), a difference of 37 times, for the pyrethrins. No figures are available for the adults.

With *T. molitor*, the most resistant stage to DDT was the pupa (middle-aged) followed by the larvae from the 6th to the 3rd size group, the adults (middle-aged) and then the 2nd and 1st size larvae. With pyrethrins, the larvae from the 6th to the 4th size groups were the most resistant followed by the pupa (middle-aged), the 3rd size larva, the adult (middle-aged) and then the 2nd- and 1st-instar larvae. The concentrations required to kill were of a lower order (0.0008 per cent., adult (old)—0.035 per cent. pupae (middle-aged)) with DDT than with pyrethrins (0.005 per cent. 1st size larva—0.2 per cent. 6th size larva) but the overall variation in resistance was approximately the same for both poisons, 44 times with DDT and 40 times with pyrethrins. No figures are available for the eggs of this species.

With *P. americana*, the most resistant stage to DDT is the 6th size nymph followed by the 5th size nymph, the adult (young), and the 3rd, 2nd and 1st size

nymphs. With pyrethrins, the 6th size nymph is also the most resistant, followed by the 5th size nymph, the 4th size nymph, adult (young), 3rd size nymph, and finally the 2nd and 1st size nymphs. No data are available on the eggs of this species. A much higher order of concentrations (0.0035 per cent. 1st size nymph—0.03 per cent. 6th size nymph) of DDT is required to kill than with pyrethrins (0.00031 per cent. 1st size nymph—0.003 per cent. 6th size nymph), but again the overall variation in resistance is similar, being 8.5 times with DDT and 10 times with pyrethrins.

These data show that large differences in resistance to insecticides occur during the development of any species. Differences of resistance between the instars ranging from about 10 to 50 times depending on the species and the insecticide have been measured, and larger differences have been shown to exist, since pupae of *D. oleracea* were resistant to a concentration of DDT 250 times that required to kill the least resistant instar. It seems likely that the magnitude of the differences that occur will depend both on the insect species and on the insecticide. Quite large differences can occur during the development of an instar, for example, the middle-aged pupa of *T. molitor* is 16.6 times as resistant as the young pupa to pyrethrins. Here also the magnitude of the difference would appear to depend on both the insect species and on the insecticide.

The majority of the comparable stages of *T. molitor* are more susceptible to DDT than to pyrethrins, while the reverse is the case with *P. americana*, thus indicating marked specificity. The data also show that the magnitude of this effect may vary with the stage of development, for the pupae of *T. molitor* are about equally resistant to DDT and pyrethrins, while the adults and larvae are considerably more resistant to pyrethrins.

From a practical point of view these data show clearly the desirability of obtaining information on the fluctuations of resistance that occur during the development of any insect pest that is to be controlled. Where a particular instar is being attacked it may be sometimes sufficient to study the changes in this instar alone, but more often a general study would appear necessary if an insecticide is to be applied intelligently and to the best advantage.

From a theoretical point of view some reasons for the changes in resistance must be sought. In the absence of detailed knowledge of the physiological, biochemical and morphological changes that occur during development, and of the mechanism of action of the insecticides, only general considerations can be put forward.

Causes of changes of resistance during development.

It seems reasonable to infer from the foregoing experiments that the resistance to poison is affected by "age" in any stage of development. While investigating the effect of different ages of eggs of *D. oleracea*, it was demonstrated experimentally that as the age of eggs, in hours, increased, the resistance to pyrethrins decreased. It was also found that a lower concentration of DDT was required to prevent emergence when older eggs were treated. Since, however, DDT did not appear capable of killing the developing embryo inside the egg shell even with the highest concentration used (0.075 per cent. w/v), but only took effect as the young larvae ate the treated chorion on emergence, it is considered that DDT is not a true ovicide and it will not be considered further. A similar increase in susceptibility to pyrethrins of tomato moth eggs with increase in age as found here was also recorded by Hough and Jefferson in the case of codling moth eggs using nicotine.

It is possible that the decrease in resistance of the eggs to pyrethrins with increase in age may be due, at least in part, to the increase in rate of metabolism of the egg with age. Increase in susceptibility following increased metabolic rate has been pointed out by Cotton (1932) and Busvine (1938). These authors, however, were discussing fumigants and their remarks may not always apply to poisons applied

Summary of the results of the experiments on the variation in resistance of the different stages of development of *D. oleracea*, *T. molitor* and *P. americana* to DDT and the pyrethrins, showing the relative resistance of the two poisons (a) on the basis of concentration required to kill a given number of individuals and (b) of the ratio of the weight of poison/weight of insect material required to give the same percentage of kill.

Stages of development and species	Age	DDT			Pyrethrins		
		LD50 DDT	Relative resistance based on concentration required to kill 50 per cent. of individuals	Relative resistance based on weight of poison/weight of insect (required to kill 50 per cent. of insect material)	LD50s total pyrethrins	Relative resistance based on concentration required to kill 50 per cent. of individuals	Relative resistance based on weight of poison/weight of insect (required to kill 50 per cent. of insect material)
Eggs of <i>D. oleracea</i>	12 hours	per cent. 0.011	1.6		per cent. 0.015	3.5	
	36 hours	0.01	1.3		0.0057	1.3	
	60 hours	0.0084	1.1		0.0044	1	
Larvae of <i>D. oleracea</i>	120 hours	0.0067	1		0.0043	1	
	1st	0.002	1.0	1	0.0027	1	1
	2nd	0.004	2.0	1.1	0.008	3	1.1
	3rd	0.0063	3.1	1.1	0.041	15	2.0
	4th	0.016	8.0	1.1	0.054	20	2.3
Larvae of <i>T. molitor</i>	5th	0.1	50	4.0	0.1	37	3.0
	1st	0.00125	1	1	0.005	1	1
	2nd	0.002	1.6	1.1	0.013	2.6	2.3
	3rd	0.0038	3.6	1.8	0.027	5.0	3.0
	4th	0.011	8.8	4.1	0.072	14.2	6.5
	5th	0.015	12.0	5.1	0.14	28.0	10.0
Nymphs of <i>P. americana</i>	6th	0.03	24.0	8.3	0.2	40.0	13.8
	1st	0.0035	1	1	0.00031	1	1
	2nd	0.0074	2.1	1.7	0.00071	2.3	10
	3rd	0.012	3.4	1.8	0.0015	4.9	10
	4th	0.017	4.8	2.0	0.0017	5.5	10
	5th	0.027	8.2	2.5	0.0021	6.7	10
Pupae of <i>D. oleracea</i>	6th	0.03	8.5	2.8	0.003	10.0	10.3
	1 day	No effect with concentrations up to 0.5 per cent. w/v				1	1
	10 days					2.9	2.9
Pupae of <i>T. molitor</i>	20 days					2.3	2.3
	1 day	0.0023	1		0.0021	1	
	4 days	0.035	15.0		0.035	16.6	
Adults of <i>T. molitor</i>	8 days	0.0076	3.2		0.007	3.3	
	1 week	0.0008	1		0.012	1	
	4 weeks	0.0029	3.6		0.022	4.1	
	8 weeks	0.0008	1		0.0054	2.2	

as contact insecticides, although Busvine's (1938) statement that "the action of poison is to dislocate the normal metabolism of an organism, and therefore, it is not surprising that the degree of poisoning is closely bound up with the physiological condition of the organism", may be regarded as a generalisation which would apply, irrespective of the mode of application of the poison. For a number of different species of insect eggs, Fink (1925), Melvin (1928), Bodine (1929) and Burkholder (1934), have shown that there is an early "formative period" which lasts a varying length of time according to the species of insect, and is characterised by a low respiratory rate. This is followed by a later period in which the metabolism increases up to the time of hatching. Smith and Pearce (1948) demonstrated that in the first two-thirds of the incubation period of codling moth eggs, the increase in respiratory activity is slight but that, beyond that point, the rate increases rapidly until hatching. From these two sets of experiments it appears that, as the age of eggs increases, the rate of respiration and metabolism increases correspondingly. If this is true it will be apparent that the decrease in resistance with increase in age of the *D. oleracea* egg to pyrethrins is at least correlated with increases in the metabolic rate.

Another possible explanation or contributory cause of the changes in resistance that have been found, may be the alteration in the structure, and possibly the permeability, of the chorion, which is known to occur as embryonic development proceeds. Beament (1949) studied the properties and permeability of sub-chorial membranes during the development of *Rhodnius prolixus* Stål. He used eggs 1-4 days, 5-7 days, 6-12 days, 13, 14, 15 days old and found that when first formed the fertilisation membrane of the egg is semi-permeable to salts and larger water-soluble particles; it was more permeable to lipophilic liquids but not larger oil-soluble molecules. During the first five to six days of incubation the epembryonic membrane, lying outside the normal living structure derived from the embryo, is added to the fertilisation membrane. The epembryonic ring and membrane constitute considerable barriers to the penetration of both lipophilic and hydrophilic substances. During the sixth and seventh days of incubation, a secondary wax layer is produced on the inside of the epembryonic membrane which further reduces the permeability to nil, and the embryo continues to develop until the twelfth day. Blastokinesis takes place and the embryo grows rapidly until it occupies the whole shell. On the 13th day the embryo apparently begins to prepare for emergence by partially dissolving the epembryonic membrane and, on the day before the egg hatches, the embryonic material is completely surrounded by a clear fluid which contains the waxy material derived from the secondary wax layer. At this stage, changes in permeability of the epembryonic membrane start again and from this point until eclosion, the egg becomes readily permeable. These changes in permeability of the egg shell of *Rhodnius* as described by Beament can be correlated with the changes in susceptibility to pyrethrins in *Diataraxia* as described in this paper.

Yet another factor in the difference in resistance with age of eggs may be the specialisation within the embryo as it develops. It is possible that pyrethrins and some other poisons are not active toxins to unspecialised cells but that, as the separate tissues become differentiated, the poison is able to exert its effect. The fact that a small percentage of the eggs were apparently killed at an early stage of development militates against this theory.

An examination of the age-susceptibility relations of the larvae of *D. oleracea* and *T. molitor* and nymphs of *P. americana* shows that the resistance increases with increase in age. These findings are in accord with those of Campbell (1926) who examined the effect of arsenic on the different instars of the silkworm and of Du Chanois (1947) who studied the effect of the gamma isomer of benzene hexachloride on the larvae of house-flies, *Musca domestica* L. When the estimate was based on the concentration required to kill a given number of individuals, Yosida (1948) found a similar relationship for the effect of pyrethrin on the larvae of *Barathra*

brassicae and *Chilo simplex* but not with *Bombyx mori*. Yosida also used his data to calculate the mean lethal concentration per unit body weight and this value decreased with increase in insect age. This method of estimating susceptibility, however, does not appear to be satisfactory since it does not take into account the changes in surface area of the insect during growth. The theory that a correlation exists between rate of metabolism and resistance to a given poison, which has been put forward when considering ovicidal effect, might apply here also. From some preliminary experiment not described in the text on the rate of respiration of the larvae of *D. oleracea* and *T. molitor* of different ages, it was observed that, as the age of the larvae increased, the rate of respiration fell; the highest metabolism, for instance, was noticed in 1st-instar *D. oleracea* larvae and *T. molitor* larvae of the smallest size (1st size). It appears therefore, that with the larvae of these two species, increase in metabolic rate as indicated by increase in rate of respiration, is correlated with increase in susceptibility to DDT and pyrethrins, judged on the concentration required to kill single individuals in each age group. This is, of course, no direct evidence that susceptibility is dependent on metabolic rate but merely enables the assumption to be made.

The problem could also be considered from the point of view of changes that occur in cuticular permeability at different stages of development. Wigglesworth (1942) demonstrated the great influence exerted by cuticle thickness on the rate of entry of pyrethrins, and the extent to which this thickness is influenced by the age and nutrition of the insect may be important factors in estimating the efficiency of pyrethrum preparations. Among other authors, Robinson (1942) and Hurst (1943) have demonstrated the slower rate of entry of pyrethrum through the presumably thicker cuticle of older insects. Klinger (1936) found a three-fold increase in the resistance of larvae of *Dendrolimus pini* (L.) during the last instar, as the endocuticle increases in thickness. Pepper and Hastings (1943) showed that in the caterpillar of *Loxostege* there is a progressive decrease in susceptibility to pyrethrum-in-oil sprays in successive instars associated, perhaps, with the progressive diminution in the fat content of the cuticle. Way (1950) has shown that the endocuticle of the larvae of successive instars of *D. oleracea* increased in thickness as the age increased. It therefore seems that there is a considerable amount of evidence to show that changes in the structure and permeability of the cuticle of larval and nymphal instars are likely to be important factors governing their susceptibility to contact poisons, but this evidence is by no means conclusive.

Passing to a consideration of the pupal stage, the experimental evidence indicated that, with both DDT and the pyrethrins, maximum resistance occurred in the middle of pupal development. When pyrethrins were used, this was found to be the case with both *D. oleracea* and *T. molitor*, but with DDT, while it was true of *T. molitor*, it was not found possible to kill the pupae of *D. oleracea* with a dose that it was possible to apply, so that no evidence is available for this species. It is noteworthy that with both insecticides and both species, whatever the concentration of insecticide and age of the pupae to which it was applied, although the resistance of the age groups differed, death did not occur until the adult was fully formed. Investigation of the respiratory rate of the pupae of various insects tends to show that the oxygen consumption and carbon dioxide output is high at first, then falls and finally rises again; this has been found by various authors, Krogh (1914) Taylor (1927) Taylor and Steinbach (1931) and Wigglesworth (1934). It seems, therefore, that with this stage also metabolic rate and susceptibility are closely correlated.

Some correlation between the data on insecticidal action and the changes that take place in the pupal cuticle during development of the pupae may be found by considering the present data in relation to the work of Wigglesworth (1948) on the pupa of *T. molitor*. Wigglesworth found that directly after pupation the pupal cuticle is soft and not fully developed, and of course no adult cuticle is present. It seems

possible that at this time the cuticle is more permeable to insecticides and this may explain the greater susceptibility that was found at this stage of development. The fact that development proceeded after application, may be explained by the fact that the pupal cuticle does not contain nervous elements. If the main action of DDT and the pyrethrins is on the peripheral nervous system it is possible therefore that the poison remains *in situ* in the pupal cuticle until the pupal peripheral nervous system has developed and then takes effect. Under the conditions of study (25°C.) that Wigglesworth employed, pupal development took eight days; after three days the adult separated from the pupal cuticle but the new cuticle of the adult had not begun to appear. After five days the eyes were fairly dark and the new cuticle well defined. In the insecticidal experiments described in this paper the middle age was taken as four days and this was found to be the most resistant period of those tested. The increase in resistance over the earlier period may be due to hardening and increase in impermeability of the pupal cuticle and the formation of an adult cuticle beneath. Wigglesworth described how at the end of the pupal period the inner layers of the pupal cuticle were digested and the moulting fluid absorbed. Since 9-day old specimens were chosen for the tests on late development pupae these changes would be taking place at the time of, or soon after, the application of the insecticide. It seems reasonable to suppose that since the pupal cuticle has been partly digested whilst the adult cuticle is still permeable to the moulting fluid, the two layers are together more permeable than in the middle period, which would account for the increase in susceptibility so clearly shown by the data.

Data is available on the adult stages of two species, *T. molitor* and *P. americana*. The experimental evidence indicated that with both DDT and the pyrethrins the changes in susceptibility were similar. Lindgren (1935) studied the respiration of *T. confusum* and found that the carbon dioxide output of old adults was very high in comparison with adults less than four days old. If rate of metabolism increases with age and also results in increased susceptibility it would provide some explanation of the results obtained with *P. americana*, but could not explain the results with *T. molitor* where the maximum resistance was shown by the middle-aged adults.

If all the data are considered it appears that increase in susceptibility can often be correlated with increased metabolic rate, although it is doubtful, in some instances at least, if this is the primary cause. In the case of eggs, larvae and pupae, change in susceptibility can be correlated with structural and permeability changes in the chorion or cuticle: again no direct evidence is available to determine if these are the critical factors. Finally, physiological and morphological changes during development, particularly with eggs and pupae, probably influence susceptibility. The reasons put forward to account for the changes in susceptibility found in the experiments are largely speculative and the supporting evidence in most cases is unsatisfactory. Further detailed work is required before any definite statement can be made.

Summary.

A review of the literature is given which shows that changes in the susceptibility of insects to insecticides occur during development when the poison is applied as a fumigant, as a stomach poison and as a contact poison.

The assessment of changes in susceptibility to contact poisons is discussed. The basis of assessment may be the concentration required to kill a given number of individuals, or the amount of poison required to kill unit weight of insect material. The great majority of the earlier work used the former basis of assessment, but where the insect is changing in size and shape during development and hence changing in the proportion of surface area to body weight, this method has limited significance when the poison is applied as a spray of constant deposit evenly over the surface area. A mathematical method is given for transforming the results obtained in terms of

concentration to kill a given number of individuals into weights of poison to kill unit weight of insect material. Rearing methods are outlined which enable batches of various stages of *Diataraxia oleracea* (L.) (tomato moth), *Tenebrio molitor* L. (meal worm) and *Periplaneta americana* (L.) (American cockroach) to be obtained at a known age and stage of development.

Experimental results are given for a spraying technique using DDT and the pyrethrins as insecticides on the comparative resistance of the eggs, larvae and pupae of *D. oleracea*, the larvae, pupae and adults of *T. molitor* and the nymphs and adults of *P. americana*.

On the basis of the concentration of insecticide required to kill a given percentage of individuals, it is shown that great differences can occur in the resistance of different instars of one species and considerable differences may occur within the instar. If the data for the larval and nymphal instars are considered on the basis of the weight of poison required to kill unit weight of insect material, differences still exist but are much reduced.

When toxicity is estimated on the basis of the concentration of poison in a constant spray deposit required to kill a given percentage of individuals the overall variations of resistance during development measured for DDT in terms of median lethal concentrations were :—*D. oleracea* eggs and larvae fifty times (0.002 per cent.—0.1 per cent. w/v), the pupa proved resistant to 0.5 per cent. w/v ; *T. molitor* larvae, pupae and adults thirty-seven and a half times (0.0008 per cent.—0.03 per cent. w/v) and *P. americana* nymphs and adults eight and a half times (0.0035 per cent.—0.03 per cent. w/v). The figures for pyrethrins were : *D. oleracea* eggs, larvae and pupae thirty-seven times (0.0027 per cent.—0.1 per cent. w/v), *T. molitor* larvae, pupae and adult forty times (0.005 per cent.—0.2 per cent. w/v), and *P. americana* nymphs and adults ten times (0.00031 per cent.—0.003 per cent. w/v).

The figures show that the range of variation of resistance during development may be very large, over 250 times in the case of DDT and *D. oleracea*, where the pupa is resistant. The maximum variation that was found within an instar was 16.6 times where the resistance to pyrethrins of the 1-day old pupa of *T. molitor* was compared with that of the 4-day old pupa. The data show that the amount of variation in resistance that can occur varies with the test species and with the insecticide. Furthermore that the order of resistance of the developmental stages of any given species will differ with the insecticide and that with any given insecticide the order will vary with the species.

It may be inferred from these data that any comparison between insecticides on one stage of development of one instar of one species will not necessarily hold true of any other stage of development of that species or of any other species.

Observations were made on the action of the insecticides on the various instars and the symptoms of poisoning are described. It was observed that DDT at the highest concentration used (0.075 per cent. w/v) failed to prevent the development of the embryo inside the egg-shell and death only occurred after the fully developed embryo had eaten the egg-shell which it normally does prior to emergence. Pyrethrins on the other hand if applied at sufficiently high concentrations could prevent embryonic development although at lower concentrations a high percentage of eggs formed fully developed embryos.

At the highest concentrations used neither DDT (0.5 per cent. w/v.) nor the pyrethrins (2.5 per cent. w/v.) were able to prevent pupal development, and partial emergence often occurred before death. DDT differed from the pyrethrins in that it was ineffective on the pupae of *D. oleracea*.

Using data given in the literature and from some preliminary experiments on respiration rates, it was possible to deduce some correlation between metabolic rate

and susceptibility, and changes in the permeability of the cuticle and chorion and susceptibility, but the evidence is unsatisfactory and the causes of the changes in susceptibility await further detailed investigation.

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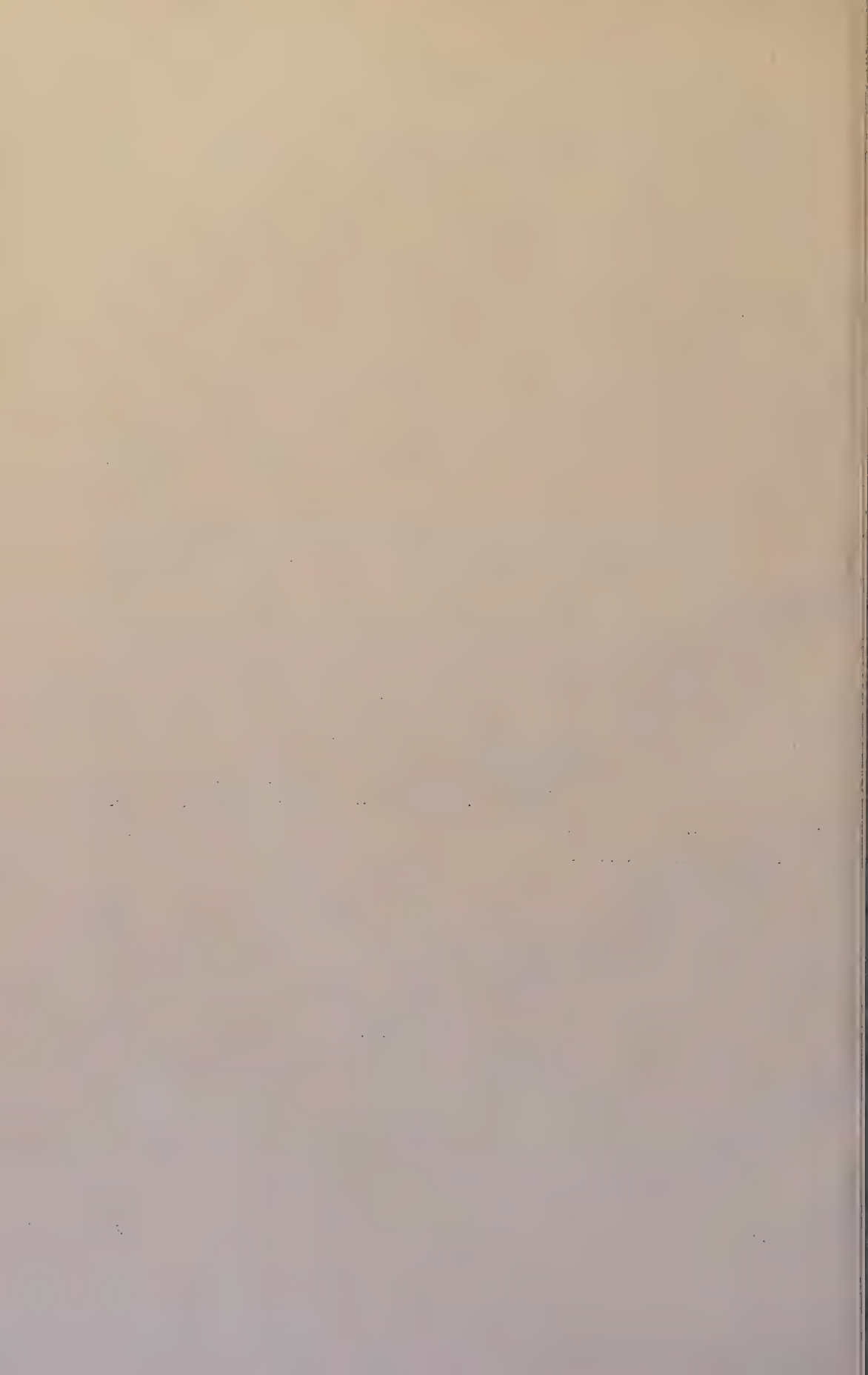
References.

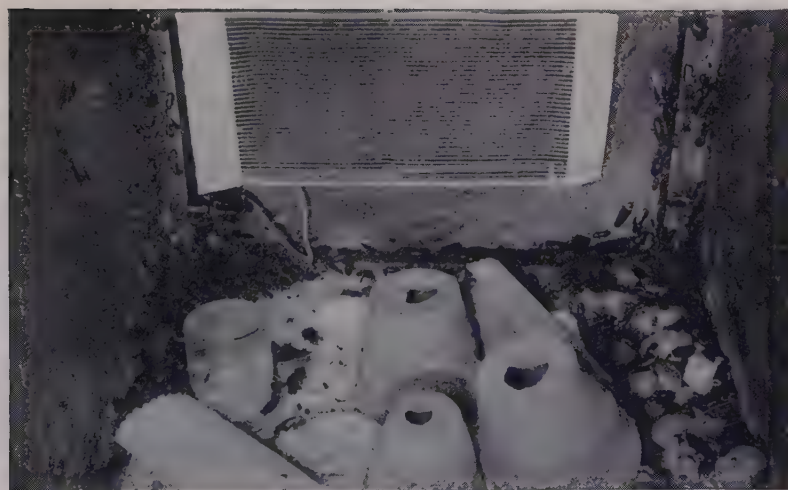
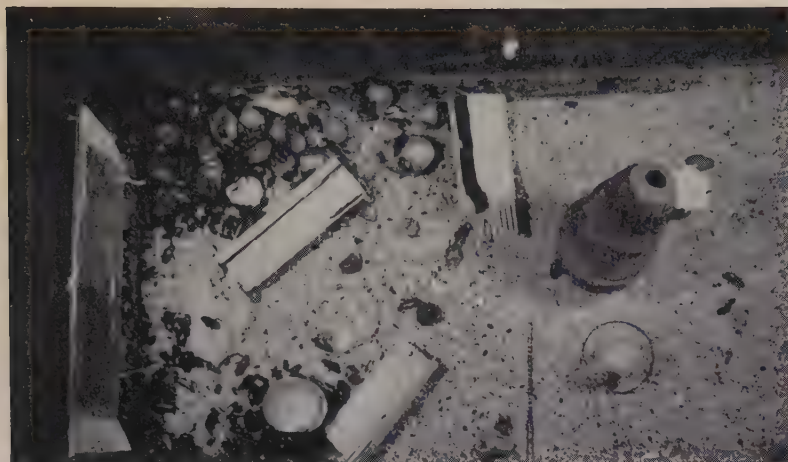
- BEAMENT, J. W. L. (1949). The penetration of insect egg-shells. II. The properties and permeability of sub-chorial membranes during development of *Rhodnius prolixus*, Stål.—Bull. ent. Res., **39**, pp. 467–488.
- BODINE, J. H. (1929). Factors influencing the rate of respiratory metabolism of a developing egg (Orthoptera).—Physiol. Zoöl., **2**, pp. 459–482.
- BURKHOLDER, J. R. (1934). A quantitative study of respiratory metabolism in single developing eggs (Orthoptera).—Physiol. Zoöl., **7**, pp. 247–270.
- BUSHLAND, R. C. (1939). Volatile oils as ovicides for the screw worm, *Cochliomyia americana*. C. & P.—J. econ. Ent., **32**, pp. 430–431.
- BUSVINE, J. R. (1938). The toxicity of ethylene oxide to *Calandra oryzae*, *C. granaria*, *Tribolium castaneum*, and *Cimex lectularius*.—Ann. appl. Biol., **25**, pp. 605–632.
- CAMPBELL, F. L. (1926). Relative susceptibility to arsenic in successive instars of the silkworm.—J. gen. Physiol., **9**, pp. 727–733.
- CHAPMAN, P. J. & PEARCE, G. W. (1949). Susceptibility of winter eggs of the European Red Mite to petroleum oils and dinitro compounds.—J. econ. Ent., **42**, pp. 44–47.
- COTTON, R. T. (1932). The relation of respiratory metabolism of insects to their susceptibility to fumigants.—J. econ. Ent., **25**, pp. 1088–1103.
- DU CHANOIS, F. R. (1947). Toxicity of gamma-benzene hexachloride to pre-imaginal stages of the housefly.—J. econ. Ent., **40**, pp. 749–751.
- FINK, D. E. (1925). Metabolism during embryonic and metamorphic development of insects.—J. gen. Physiol., **7**, pp. 527–543.
- GOUGH, H. C. (1939). Factors affecting the resistance of the flour beetle, *Tribolium confusum* Duv., to hydrogen cyanide.—Ann. appl. Biol., **26**, pp. 533–571.
- GUNDERSON, H. & STRAND, A. L. (1939). Toxicity of hydrogen cyanide, chloropicrin and ethylene oxide to eggs, nymphs and adults of the bed bug.—J. econ. Ent., **32**, pp. 106–110.
- HARRIES, F. H., DECOURSEY, J. D. & HOFMASTER, R. N. (1945). Some factors affecting the insecticidal action of pyrethrum extracts on the beet leaf-hopper.—J. agric. Res., **71**, pp. 553–565.
- HOUGH, W. S. & JEFFERSON, R. N. (1936). Tests of insecticidal efficiency of some contact sprays against codling moth eggs.—J. econ. Ent., **29**, pp. 537–541.

- HURST, H. (1943). Principles of insecticidal action as a guide to drug reactivity phase distribution relationships.—Trans. Faraday Soc., **39**, pp. 390–411.
- *HUTZEL, J. M. (1942). Action of pyrethrum upon the German cockroach.—J. econ. Ent., **35**, pp. 933–937.
- *KLINGER, H. (1936). Die insektizide Wirkung von Pyrethrum—und Derrisgiften und ihre Abhängigkeit vom Insektenkörper.—Arb. physiol. angew. Ent. Berl., **3**, pp. 49–69, 115–151.
- *KROGH, A. (1914). On the rate of development and CO₂ production of chrysalides of *Tenebrio molitor* at different temperatures.—Z. allg. Physiol., **16**, pp. 178–190.
- LINDGREN, D. L. (1935). The respiration of insects in relation to the heating and the fumigation of grain.—Tech. Bull. Minn. agric. Exp. Sta., no. 109, 32 pp.
- LUDWIG, D. (1946). The effect of DDT on the metabolism of the Japanese beetle, *Popillia japonica* Newman.—Ann. ent. Soc. Amer., **39**, pp. 496–509.
- MCGOVAN, E. R. & FALES, J. H. (1942). Roach testing.—Soap & sanit. Chem., **18**, no. 3, pp. 101, 103, 105, 107, 117.
- MCINTOSH, A. H. (1947). Relation between particle size and shape of insecticidal suspensions and their contact toxicity. I. DDT suspensions against *Tribolium castaneum* Hb.—Ann. appl. Biol., **34**, pp. 586–610.
- *MELVIN, R. (1926). Oxygen consumption of insect eggs.—Biol. Bull., **55**, pp. 135–142.
- NEWCOMMER, E. J. & YOTHERS, M. A. (1932). Experiments with insecticides for codling-moth control.—Tech. Bull. U.S. Dep. Agric., no. 281, 28 pp.
- PARKER, B. M. & CAMPBELL, F. L. (1940). Relative susceptibility of the oötheca and adult female of the German cockroach to liquid household insecticides.—J. econ. Ent., **33**, pp. 610–614.
- PEPPER, J. H. & HASTINGS, E. (1943). Age variations in exoskeletal composition of the sugar beet webworm and their possible effect on membrane permeability.—J. econ. Ent., **36**, pp. 633–634.
- POTTER, C. (1952). An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomised spray fluids.—Ann. appl. Biol., **39**, pp. 1–28.
- POTTER, C. & GILLHAM, E. M. (1946). Effects of atmospheric environment, before and after treatment, on the toxicity to insects of contact poisons. I.—Ann. appl. Biol., **33**, pp. 142–159.
- POTTER, C. & TATTERSFIELD, F. (1943). Ovicidal properties of certain insecticides of plant origin (nicotine, pyrethrins, derris products).—Bull. ent. Res., **34**, pp. 225–244.
- ROBINSON, G. G. (1942). The penetration of pyrethrum through the cuticle of the tick *Ornithodoros moubata* Murray (Argasidae).—Parasitology, **34**, pp. 113–121.
- SIMANTON, W. A. & MILLER, A. C. (1937). Housefly age as a factor in susceptibility to pyrethrum sprays.—J. econ. Ent., **30**, pp. 917–921.
- SMITH, E. H. & PEARCE, G. W. (1948). The mode of action of petroleum oils as ovicides.—J. econ. Ent., **41**, pp. 173–180.
- SUN (YUN-PEI). (1947). An analysis of some important factors affecting the results of fumigation tests on insects.—Tech. Bull. Minn. agric. Exp. Sta., no. 177, 104 pp.

- TATTERSFIELD, F. & POTTER, C. (1943). Biological methods of determining the insecticidal values of pyrethrum preparations (particularly extracts in heavy oil).—Ann. appl. Biol., **30**, pp. 259–279.
- TAYLOR, I. R. (1927). Oxygen consumption of individual pupae during metamorphosis.—J. Morph., **44**, pp. 313–339.
- TAYLOR, I. R. & STEINBACH, H. B. (1931). Respiratory metabolism during pupal development of *Galleria mellonella* (bee moth).—Physiol. Zoöl., **4**, pp. 604–619.
- *TOBIAS, J., KOLLROS, J. & SAVIT, J. (1946). Relation of absorbability to the comparative toxicity of DDT for insects and mammals.—J. Pharmacol., **86**, pp. 287–293.
- TUMA, V. (1938). Roaches. A study of the relationship between the ages of cockroaches and their resistance to insecticides.—Soap & sanit. Chem., **14**, no. 6, p.p. 109–111, 113, 115, 117, 151.
- WAY, M. J. (1950). The structure and development of the larval cuticle of *Diataraxia oleracea* (Lepidoptera).—Quart. J. micr. Sci., **91**, pp. 145–182.
- WAY, M. J., HOPKINS, B. & SMITH, P. M. (1949). Photoperiodism and diapause in insects.—Nature, Lond., **164**, p. 615.
- WAY, M. J., SMITH, P. M. & HOPKINS, B. (1951). The selection and rearing of leaf-eating insects for use as test subjects in the study of insecticides.—Bull. ent. Res., **42**, pp. 331–354.
- WIGGLESWORTH, V. B. (1934). Insect physiology, p. 78. London, Methuen.
- WIGGLESWORTH, V. B. (1942). Some notes of the integument of insects in relation to the entry of contact insecticides.—Bull. ent. Res., **33**, pp. 205–218.
- WIGGLESWORTH, V. B. (1948). The structure and deposition of the cuticle of adult mealworm, *Tenebrio molitor* L. (Coleoptera).—Quart. J. micr. Sci., **89**, pp. 197–217.
- WOODBURY, E. N. (1938). Test methods on roaches.—Soap & sanit. Chem., **14**, no. 8, pp. 86–90, 107, 109.
- YOSIDA, M. (1948). Toxicity of pyrethrin to certain insect larvae at their different stages of growth. [In Japanese with an English summary.].—Botyu-Kagaku, no. 10, pp. 60–68.

* References seen as abstracts only.





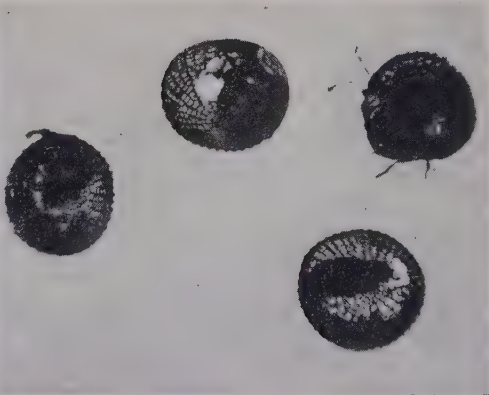
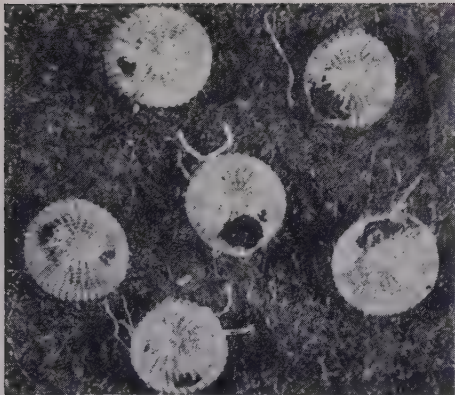
Interior of the stock cage for breeding all stages of *Periplaneta americana* (L.). Note the pieces of pleated paper and flower pots placed in piles serving for a hiding place for adults and nymphs. The thermostatic control device and heater are also shown.



Examples of the six size groups used in the tests on larvae of *Tenebrio molitor* (L.).



Examples of the six size groups used in the tests on nymphs of *Periplaneta americana* (L.).



Showing the first incubation poisoning effect of DDT on the eggs of *Diataraxia oleracea* (L.). Note the outline of the dead bodies of the larvae visible through the chorion; also that the egg shell has been eaten prior to death.

ON SWARMING AND MATING IN *ANOPHELES AQUASALIS* CURRY.

By R. A. SENIOR WHITE, G. LEWIS and P. LEE.

Entomological Section, Malaria Division, Health Department, Trinidad.

Senior White (1951), the first author of the present paper, published some preliminary findings on swarming and mating in *Anopheles aquasalis* Curry, stating that from 1951 onwards a more detailed study of these habits was being undertaken.

The conclusions reached as the result of these first studies were :—

- (a) that swarm formation followed sunset at a lag averaging 20 minutes,* and occurred at a light value not exceeding 5 foot-candles ;
- (b) that females, though not rare in the swarms, were very few in numbers compared with the males ;
- (c) that females captured in the swarms were invariably unfed, but that a high percentage were already fertilised ;
- (d) that considering the vast *aquasalis* population of the area studied, the swarms did not appear to be the main site of fertilisation, which therefore remained unknown.

Detailed and continued observational work was carried out in Trinidad all through 1951, and the results of this form the subject of the present paper. The second author led the observing parties during the first author's absence on furlough from mid-April to mid-July, 1951. During this period the photometer was not in use.

A tabulation of the literature (Table I) on *natural* swarming and mating in a number of *Anopheles* spp. is given, from which a comparison can be instituted in respect of the behaviour-pattern of *aquasalis* with other species of the genus. It will be seen that the performance of the two acts are quite different as between all the species so far studied, so that no generalisations can be advanced. Every species appears to have its own behaviour pattern.

Cage mating of *A. albimanus* Wied., *A. darlingi* Root, *A. elutus* Edw., *A. fluviatilis* James, *A. funestus* Giles, *A. gambiae* Giles, *A. maculipalpis* Giles, *A. maculipennis* Mg., *A. mauritanus* Grp., *A. multicolor* Camb., *A. pseudopunctipennis* Theo., *A. quadrimaculatus* Say and *A. stephensi* List. has been obtained by various workers, but this is not germane to the natural act, which in these species seems never to have been observed. Equally, the present authors have so far failed to obtain definite proof of cage mating with *aquasalis*, even in an insectary 40×20×10 ft. high. As will be noted in the Table, it has been assumed that mating in an insectary of such dimensions is "natural", as it is really an enclosure of a natural area. Nonetheless, some of the swarming heights observed with *aquasalis* would be inhibited by a 10 ft. high roof. The insectary used by Hackett and Bates (1938) in Albania was twice this height, yet within it *maculipennis* (s.l.) and *subalpinus* Hack. & Lewis refused to swarm, though with the former species, mating, as with *aquasalis*, must have occasionally occurred, as a few fertile eggs have been found in both insectaries. Bates' 1941 paper was primarily concerned with cage breeding, and in it observations on the natural act are scattered and somewhat difficult to follow.

* The time of sunset has been taken from the Nautical Almanac as that for 10°N. latitude. The Caroni Plain of Trinidad, the scene of our work, is about 10°38'N. latitude, so actually the time of sunset is not that given in the Almanac but a few minutes later.

TABLE I.
Résumé of data on natural swarming in *Anopheles* spp.

Species	Light at start of swarming f.c.	Numbers in swarm	Orientation of swarm	Position of swarm	Mating	Notes	Author
<i>annularis</i> ...	0.9-2.3	5-30	Into wind	Overhead on sheltered hard tennis court	Seen ...	—	Ramachandra Rao & Russell (1938).
<i>atoparvus</i> ...	10-15	Dense	Into wind	—	With resting ♀♀	—	Cambournac & Hill (1940).
<i>bifurcatus</i> ...	—	20-100	Facing moors where no breeding	Bays in forest edge—"open" in warm weather	Seen ...	—	Marshall (1938).
<i>culicifacies</i> ...	2-6	100-150	East not into wind	Over dry area with no obstructions	Seen ...	In insectary 40 × 20 × 10 ft.	Russell & Ramachandra Rao (1942).
<i>franciscanus</i> ...	—	up to 5,000	—	Over roof; over bridge rail	Seen ...	—	Belkin & others (1951).
<i>messeae</i> ...	"Just after Sunset"	Dense	—	Over low tree or hedge	—	—	Marshall (1938).
<i>funestus</i> ...	—	300-500	—	Threshold of thatched hut	Not seen ...	—	Harper (1944)
<i>gambiae</i> ...	—	small	—	Over bush at 6-7 ft.	Not seen ...	—	Muirhead Thomson (1948).
<i>plumbeus</i> ...	—	4-5	—	In open near moator meadows	—	—	Marshall (1938).
<i>p. pseudopunctipennis</i>	—	—	—	Over tree and house-tops also near ground	In exit trap, not in nature	? Unfed ♀♀ resting in house unmated, normally going out to mate and return	Bordas & Downs (1951).
<i>subpictus</i> ...	—	45	—	Sheltered hard tennis court	—	Only once observed; 1 ♀, and a number of ♂♂ taken, determined on ♀	Ramachandra Rao & Russell (1938).
<i>superpictus</i> ...	1-15	Dense	Wind (fan) disperses	In stable 1-2 ft. over dung; 5 ft. above stable roof	—	Responds to change in light + or -	Bates (1941).

Data on swarming in *aquasalis* have now been obtained over 133 evenings. The majority of the observations have been made round the experimental bush quadrat in Bordenal Savannah described in Senior White (1951 Section VIII), but observations have been carried out at many points over the ten-mile strip from Caroni Savannah near the Imperial College of Tropical Agriculture to the seaface,* just east of Port of Spain. Whilst swarms have been seen in many localities (*vide infra*), and at several spots adjacent to this quadrat, it was found that there was one spot near the quadrat which was almost invariably chosen for the act. This was over the grass that surrounds the scrub near the south-west post of the boundary fence. Evening after evening a swarm could be seen about two feet south of this post, over a grass-covered drain which here separates the Division's experimental plot from land to the west which is, during the rains, under rain-fed rice, and subsequently under vegetables. There seems to be a definite association between drains, whether dry or wet, and swarming points. The whole of Bordenal Savannah was once sugar-cane land and it is therefore criss-crossed by old drains. Most of them are now silted up to within a foot of the general surface. They have few outfalls, and periodically fill up with rainwater that slowly percolates into the soil. But it is not every drain that is chosen as a swarming site.

The exact venue of the swarm at this south-west corner of the quadrat is not absolutely fixed. Whilst this point has never been observed to move westward over the open rice-land, the exact swarming point may move by as much as six feet eastward of the post, parallel to the bush face of the quadrat and the boundary wire. In the vicinity other swarms are at times seen over other drains, particularly one running beneath a field boundary hedge of *Cordia macrostachya* and other medium

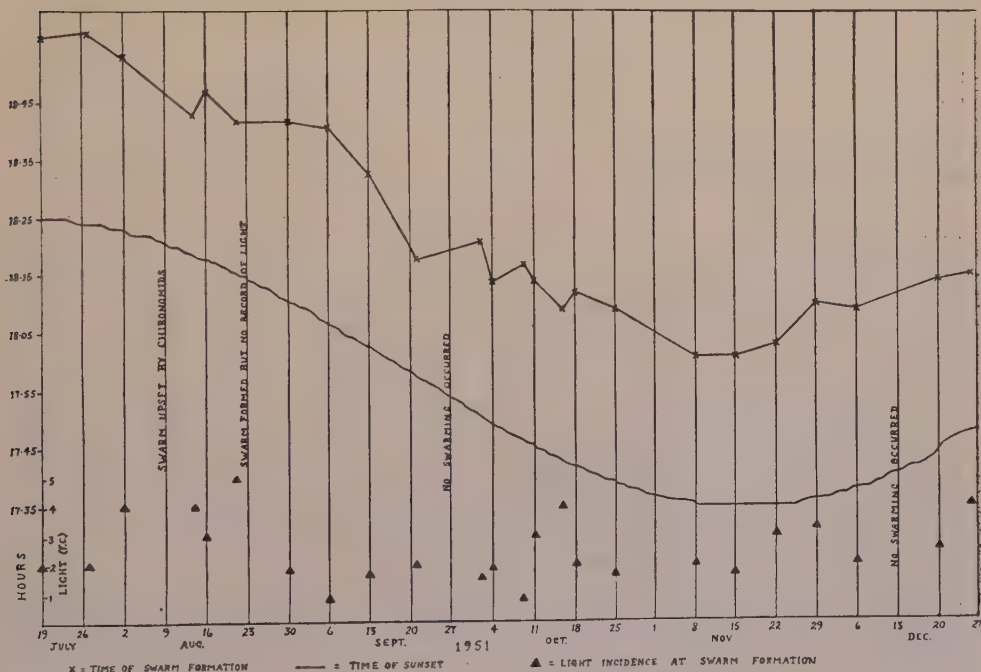


Fig. 1.

*The term "seaface" is used in preference to "seashore" because the mangrove at this point extends into the open sea and there is no actual shore.

height scrub. Swarms have also been seen over grass well away from bush, and on the lee (west) side of the experimental hut used in earlier experiments described by Senior White (1951), where the same drain passes as that from the south-west corner post. Just east of this hut, and about 30 ft. south-east of the south-west corner post, where another drain has been deepened to keep the area of the quadrat dry, swarming has never been seen, and it has thus been possible to make psychrometric observations simultaneously at the swarming point and at this point of no-swarming. Light readings were made midway between the two points, with the photometer target horizontal, supported above the grass boundary to the bush quadrat.

Table II is an abstract of the observations at this regular spot over a year, and fig. 1 is a plot of some of these results, showing the sunset curve, and time and light-incidence at swarm formation over this period. There is obviously a high degree of correlation between time after sunset and of swarm formation, but Table I shows that the time-lag is not fixed. On the same night, in the same vicinity, swarms do not form and disappear absolutely synchronously, but though this may partly be due to greater acuity of perception with one observer than with another, yet there are undoubtedly differences of as much as ten minutes between the time of formation of individual swarms in one neighbourhood.

The formation of a swarm is initiated by a few males circling rather widely apart at a height varying from 6 to 15 ft. Other males quickly join these pioneers, and a true swarm is formed, which may, on a "good" evening, increase to an estimated number of 600-800. Like Nielsen and Greve (1950), whose work on swarm formation in *Aedes cantans* (Mg.) and *A. communis* (Deg.) forms the only previous extensive study on this subject, we have not succeeded in netting an entire swarm for an exact count of numbers. The largest number netted on a single night was 648♂♂, 5♀♀ (together with 19♂ *Anopheles oswaldoi* Peryassú) in 14 sweeps of an 18-inch diameter net on a long stick, which is an awkward instrument to handle. Sweeping in the swarm on six nights did not indicate a larger female percentage than two, but sweeping so disturbs the swarm, even though it reforms, that the practice was abandoned as a measure of density, though it has been carried out every time a swarm is seen in order to check the species concerned. Observations on behaviour were preferred to such crude counting methods, so the formed swarm was not further disturbed.

When fully formed, the swarm forms a column, often more diffuse basally than higher up, several feet in height, the top frequently exceeding the 10 ft. height of a graduated pole used for suspending a psychrometer at this spot. The Bordenal quadrat swarm seldom had its base lower than 4 ft. above ground level, but elsewhere we have seen swarms at no more than 2-3 ft. above ground level. So far as can be seen in a fading light, males appear to face north-easterly, *i.e.*, across the very light wind usually prevailing after sunset, and nearly "tail on" to the sunset. A just perceptible air-movement seems to favour swarm formation more than a dead calm.

At first we believed that the presence of an animal tethered nearby was necessary to induce a swarm. Certainly swarms have been seen to form over an animal, or an observer, and in the case of a man to rise or fall according to whether he stood up or squatted, and we therefore deduced a connection between swarm formation and the column of slightly warmer rising air over an animal, but this procedure was soon abandoned as unnecessary. To observe swarm formation in a low light the close presence of one or more observers is required and from such warmed air must necessarily be rising, but it is no longer believed that there is a close connection between convectional air movement and a swarm, for were this so, a swarm would always have been formed over the first author, who, throughout these observations, has been seated, with the photometer and a notebook, at a camp table. Swarming appears to be related to fixed points, and appears at such even when an animal is tethered a few feet away.

A swarm can be temporarily broken up by coughing, hand-clapping or shouting in its vicinity or by gusts of wind. It can also be broken up by placing a light-coloured sheet beneath it, or by throwing a torch-beam on to the subjacent ground. The second author found that by directing a torch beam to the ground beneath it, a swarm can be driven along the course of, or even a few feet to the side of a drain.

The record of the time at which a swarm is lost to sight is partially dependent upon the state of the sky, as well as upon the acuity of vision of the observer ; observation is much more difficult against dark cloud than a clear sky.

From the constant formation of a swarm at one particular spot, and its occasional formation, at, and also complete absence from other nearby spots, swarm formation appears to be governed by some physical factor, probably meteorological. We have attempted to evaluate this factor by simultaneous psychrometer readings at pairs of contrasting points :—

- (i) at ground level beneath a swarm and at the same level on grass when there was no swarm ;
- (ii) at 6 ft. in the same spots ;
- (iii) at 1 ft. and 6 ft. at a swarming point ;
- (iv) at 6 ft. in a swarm over grass and at 6 ft. over tall scrub 6 ft. distant where swarms never form save on the occasions when an observer for the psychrometer has been in the scrub.

According to the weather of the evening, each series of simultaneous observations gave widely different results at the start, but *at the time of swarm formation*, as between any pair of points, we obtained the following readings of saturation deficiency (in mm.) :—

- (i) At ground level beneath a swarm, and where no swarm. No appreciable differences in saturation deficiency recorded.
- (ii)* At 6 ft. over grass beneath a swarm and at 6 ft. where no swarm : 2.1 and 2.7 : 4.1 and 3.8 : 4.0 and 4.0. Average 3.4 and 3.5.
- (iii)* At 1 ft. and 6 ft. at a swarming point : 0.8 and 2.2 : 2.0 and 2.0 ; Average 1.4 and 2.1.
- (iv)* At 6 ft. in a swarm over grass and 6 ft. over tall scrub, 6 ft. distant where swarms never form save on occasions when an observer for the psychrometer has been in the scrub. Their formation on these occasions suggests that convection does play some part in swarm formation : 4.8 and 3.4 : 1.9 and 1.0 ; 1.7 and 1.5. Average 1.8 and 1.0.

In addition, readings were taken at ground level and at 10 ft. (*i.e.*, above the microclimate levels used by Geiger) with the following results, which serve to show that there are differences at 10 ft. which greatly exceed the top of the microclimate at 6 ft.

Ground level and 10 ft. : 1.0 and 3.8 : 0.5 and 3.0 : Average 0.7 and 3.4.

The differences between a given pair of points on individual nights may then be appreciable, up to 1.4 mm., or it may be nil. The readings at 0 ft. and 10 ft. differ more widely.

Elaborate as their meteorological set-up was, involving complex electrical circuits, Neilsen and Greve (1950) failed to measure dry and wet bulb differences.

* The elevation of 6 ft. was chosen since Geiger (1950) states that 1.5 metres marks the limit between conditions considered in microclimatology and those measured in the standard screen. It is below the usual height of initiation of swarming in *aquasalis*. Geiger points out that the air drawn in by the fan of an Assmann psychrometer comes, on an average, from a layer 4 cm. higher than that of the mouths of the suction tubes.

TABLE II.
Bordenal Quadrat : Observations on *A. aquasalis* swarms near south-west fence-post over grass, 1951.

Date	Sunset for 10°N.	Swarm seen	Min. after sunset	Light f.c.	Height of swarm, ft.	Approx. nos. in swarm at maximum	Mating seen	Time lag swarm-mating	Light f.c.	Swarm lost	Min. after sunset	Light f.c.	Duration of swarm, min.	Remarks
Jan. 4	17.51	18.07	16	—	—	very large	Yes	—	—	18.29	38	—	22	Breeze disturbing swarm.
" 18	17.59	18.17	18	—	3	very large	Yes	6	—	18.41	42	—	24	
" 19	17.59	18.18	19	—	8-2	very large	Yes	10	—	18.39	40	—	21	
" 23	18.01	18.17	16	—	10-5	very large	Yes	4	—	18.39	38	—	22	
" 30	18.04	18.18	14	—	10-7	large	Yes	4	—	18.38	34	—	20	
" 31	18.04	18.21	17	—	12-6	large	No	—	—	18.42	38	—	21	
Feb. 1	18.05	18.18	13	—	12-5	large	Yes	2	—	18.40	35	—	22	
" 9	18.07	18.22	15	—	—	large	Yes	18	—	18.40	33	—	18	
" 15	18.08	18.22	14	—	12	very small	Yes	10	—	18.39	31	—	17	
" 22	18.10	18.23	13	—	—	very small	Yes	10	—	18.44	34	—	21	Gusty—swarm disturbed.
Mar. 1	18.10	18.27	17	4	8	not large	Yes	1	3	—	—	—	—	
" 8	18.11	18.31	20	3	7	small	Yes	4	1	18.38	27	20-35	7	Possibly swarm moved 18.38.
" 15	18.11	18.25	14	5	10-4	large	Yes	8	—	18.45	34	0-01	20	♀♀ released to watch mating.
" 21	18.11	18.28	17	5	9	not large	Yes	5	—	18.47	36	20-01	19	♀♀ released to watch mating.
Apr. 5	18.10	18.28	18	2	10-8	large	No	—	—	18.47	37	0	19	
" 12	18.10	18.37	21	2	15	small	No	—	—	18.45	35	0-04	14	
" 19	18.11	18.37	26	1	—	very small	—	—	—	—	—	—	—	No swarm formed.
" 23	18.11	—	—	—	—	—	—	—	—	—	—	—	—	No swarm formed.
" 26	18.11	—	—	—	—	—	—	—	—	—	—	—	—	No swarm formed.
May 23	18.15	—	—	—	—	—	—	—	—	18.35	18	—	8	No swarm formed. This observation time is doubtful.
" 31	18.17	18.20	12	—	—	—	—	—	—	—	—	—	—	No swarm formed.
June 8	18.20	18.45	25	—	5-8	200	No	—	—	19.09	49	—	24	No swarm formed. heavy ground mist.
" 14	18.21	—	—	—	—	—	—	—	—	—	—	—	—	
" 21	18.23	—	—	—	—	—	—	—	—	—	—	—	—	
" 28	18.24	18.45	21	—	14-10	400	Yes	—	—	19.00	36	—	15	
July 6	18.25	18.45	20	—	14-10	550	Yes	—	—	19.01	36	—	19	
" 13	18.25	18.44	19	—	10-4	—	Yes	—	—	19.03	38	—	19	
" 19	18.25	18.46	21	2	11-10	300	Yes	0	2	19.02	37	0-01	16	Swarm and mating simultaneous.
" 27	18.24	18.47	23	4	12-5	large	Yes	6	0-4	19.08	44	0	21	
Aug. 2	18.23	18.43	20	4	15-5	very large	Yes	7	0-6	19.04	41	0-01	21	Swarm formation time upset by Chironomid swarm. Swarm dense at 18.43. Mating seen 18.45.
" 9	18.20	—	—	—	15	large	Yes	?	?	18.54	34	0-01	?	
" 14	18.18	18.33	15	4	15-8	very large	Yes	4	3	18.52	34	20-05	19	
" 16	18.18	18.37	19	3	12-7	very large	Yes	3	1-2	18.57	39	0-02	20	
" 21	18.15	18.32	17	5	12	very large	Yes	13	0-15	18.53	38	0	21	3♂ flying around 18.35.
" 23	18.14	18.29	15	?	18-5	very large	Yes	8	0-8	18.50	36	0-01	21	
" 30	18.11	18.31	20	1-3	13-8	200	Yes	4	0-5	18.48	37	0	17	Swarm unusually diffuse.

TABLE II. (cont.)
Bordenal Quadrat : Observations on *A. aquasalis* swarms near south-west fence-post over grass, 1951.

Date	Sunset for 10°N.	Swarm seen	Min. after sunset	Light f.c.	Height of swarm, ft.	Approx. nos. in swarm at maximum	Mating seen	Time lag swarm-mating	Light f.c.	Swarm lost	Min. after sunset	Light f.c.	Duration of swarm, min.	Remarks
Sept. 6	18.06	18.31	25	0.8	15-6	100	Yes	4	0.5	18.44	38	0.02	13	1st ♂ circling 18.30. Swarm unusually diffuse. Only 2 mating pairs seen. Much mating for a small swarm. About 24 pairs counted.
" 13	18.02	18.23	21	1.7	8-6	100	Yes	4	0.5	18.37	35	0.01	14	No true swarm. 1 ♂ in Chironomid swarm.
" 19	17.58	18.27	29	0.2	—	very small	No	—	—	—	—	—	—	Drizzling. 1 ♂ circling 18.14. 18.23 a few males circling. No true swarm. None catchable for determination.
" 21	17.57	18.08	11	2	15-8	large	Yes	12	?	18.30	33	0	22	Swarm unusually diffuse. Fertilised ♀♀ released with ♂♂ nearby. Rather strong breeze.
" 27	17.54	18.14	20	2	10	very small	No	—	—	—	—	—	—	1 ♂ circling 18.03. Swarm diffuse. Rain. Swarm lost while still light. Swarm dispersing from 18.11.
Oct. 2	17.50	18.11	21	1.5	8-6	not large	Yes	10	0.6	—	—	—	—	Nearby swarm coalescing and separating. Light drizzle.
" 4	17.49	18.04	15	1.9	10-8	100	No	—	—	18.18	29	0.04	14	Swarm scattered after 18.02.
" 9	17.46	18.07	21	0.8	8-6	100	No	—	—	18.14	28	?	7	None catchable for determination.
" 11	17.45	18.04	19	3	11	200	No	—	—	18.17	32	0.06	13	Gusty swarm being scattered.
" 16	17.43	17.59	16	4	12-10	400	Yes	11	0.25	18.20	27	0.02	21	Mating pairs seen below swarm.
" 18	17.42	18.02	20	2	12-6	200	Yes	6	0.4	18.21	39	<0.01	19	Heavy attack 5 minutes before swarm seen.
" 25	17.39	17.59	20	1.7	11-9	150	Yes	7	0.25	18.11	32	0.04	12	Wind scattering 3 minutes after formation. 1 mating pair at 18.00 at 8 ft.
Nov. 1	17.37	18.03	26	0.4	10	?	No	—	—	—	—	—	—	Gusty with light drizzle.
" 8	17.35	17.51	16	2.4	15	400	Yes	8	0.6	18.10	35	0.02	19	
" 15	17.35	17.52	17	1.7	11	40	No	—	—	18.06	31	0.02	14	
" 22	17.35	17.53	18	3.0	12-9	200	Yes	—	—	18.03	34	0.04	16	
" 29	17.36	18.00	24	3.2	—	very small	No	—	—	18.10	34	0.01	10	
Dec. 6	17.38	17.59	21	2.0	12-9	100	No	—	—	18.14	36	0.04	15	
" 13	17.40	?	?	?	?	?	Yes	?	0.3	18.00	—	—	—	
" 20	17.44	18.04	20	2.5	10	75	Yes	5	0.6	18.19	35	0.06	15	
" 27	17.47	18.05	18	4.0	12-8	small	No	—	—	18.23	36	0.04	18	
Mean			19.0	2.5				6.5	0.83				18.0	
S.D.			3.9	1.21				3.6	0.008				1.42	
S.E.			0.55	0.04				0.68	0.002				0.21	

During our observations air temperature differences found were :—

Ground level and 10 ft. : 2.0° and 2.5°.

1 ft. and 6 ft. : 1.5° (two readings).

6 ft. over grass and over scrub : 0° and 0.5°.

6 ft. over grass where swarm and no swarm : 0° and 0.5°.

Such small temperature differences in the lowest 6 ft. of air can hardly be looked upon as any more significant than those found in saturation deficiency. If swarm formation is dependent upon differences in either of these elements, measurements must involve the use of far more delicate instruments than are available to us. An attempt to induce swarming at a different spot by putting out a tin of hot water to induce a heat gradient evoked no response.

Extended observations have only served to confirm our earlier work, and that of all other observers of swarming, that formation is governed by light incidence. On no occasion throughout the year has light at this moment exceeded the previously published figure of 5 foot-candles for *aquasalis*, and the Table shows that the mean light value is 2.5 foot-candles. This value leaves only a few minutes of visibility to observe happenings after swarm formation.

A few observations at dawn have yielded no positive results, and as Senior White (1951) shows, with some half of the total *aquasalis* female activity of the night confined to the first 2½ hours of darkness, it is possible that this species does not swarm around dawn.

Regarding the total amount of swarming as explanatory of fertilisation of the vast *aquasalis* production of the Caroni Plain of Trinidad, a good many cruising observations have been made. So long as one avoids the vicinity of houses, where swarms of *Culex fatigans* Wied. originating from cess-pits are frequent and confusing, and observation is made over grass and *not* scrub, with a sky background—for nothing can be seen against a background of trees—swarms have been seen everywhere except over the stretch of *Acroceras-Paspalum* verge between the mangrove face and the Eastern Main Road along the railway into Port of Spain. Before this stretch was levelled up and drained in connection with other experiments, there was heavy breeding in this area. Here observers are attacked, but swarming has never been seen. Chironomid swarms originating from concrete sullage drains are common and confusing. It was not until *aquasalis* breeding had been eliminated from this stretch that swarming was looked for within the actual mangrove. On two out of four evening visits to the mangrove, swarming was seen in an open area, described in Senior White (1951), with scattered bushes of *Avicennia* and numerous young *Rhizophora* plants, but in the main consisting of bare mud with a subaqueous growth of *Ruppia maritima*. On the two occasions when swarm formation (and mating) had been seen here, the area was tide-submerged to about 12 in. and on the two occasions when no swarm was seen the depth of water did not exceed 4 inches. All four observations were made over a period of three weeks. Michelmores (1947) has shown that over a lake the air may be far from saturated some distance from shore, but we have made no instrumental observations in this mangrove : movement in it after dark is far too hazardous a procedure to permit carrying delicate instruments.

No swarm has been recorded as *aquasalis* until a net sweep within it has definitely taken the species, or this was caught in a nearby swarm. In the fading light swarms of several species of Chironomids, small Tipulids and particularly of male ants are very liable to deceive the observer. Swarms of these species may form contiguous to those of *aquasalis*. As recorded in the first paper, *albitarsis* and *oswaldoi* have been taken on one occasion each in sample nettings, when *aquasalis* has formed part of the same sweep. A swarm of *neomaculipalpus* Curry has once been seen over a water-filled drain two miles west of Bordenal quadrat, but nowhere else, and on no other

occasion, though the species is generally distributed up to within two miles of the seaface. It was on that occasion separate from a nearby swarm of *aquasalis*, and the dancing movements of the males forming it were not as rapid as in that species. The swarm was dispersed by the sampling sweep, and did not reform. Swarming of *albitarsis* was only observed on three occasions, in December 1951. It seems to occur at about 3–4 ft. elevation over grass. Mating in these swarms has not yet been observed. Formation has occurred at 0.4 to 0.1 foot-candles, and the swarm cannot be seen for more than a very few minutes in the last of the light. Swarms of undetermined *Culex* spp. and on a few occasions a pair or two of dancing males of *Mansonia* ? *titillans* (Wlk.) have been seen.

Turning now to whether these male swarms are the sole or the principal site of fertilisation of the females: out of 121 swarms watched, mating has been observed in 65 (54 per cent.) of them. In the case of Bordenal quadrat corner-post swarm, in 68 per cent. of 50 observations. Including the records made whilst the first author was absent in April–July, when the second author's parties observed many swarms to which detailed attention on this matter was not always paid by individual assistants who were out of his immediate view, it would appear that the probability of observing mating is lower in some months than in others, these latter being those of the dry season months, when breeding is minimal.*

Month	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	Total
Swarms seen	7	4	6	20	17	9	16	15	6	7	9	5	121
Matings seen	5	4	5	3	5	2	12	12	4	4	6	3	65
Per cent. swarms mating ...	71	100	83	15	29	22	75	80	67	57	67	60	54

A. aquasalis has never ceased breeding in the observational area since the spring drought of 1949, but the percentage of difference between these and other months of the years of this study is so large as to suggest that paucity of mating in swarms during the dry season is in fact related to scanty breeding (see also footnote).

Observations on mating are difficult, as at the time it occurs the light has nearly gone. A pair *in copula* can however be distinguished by their larger appearance than that of an individual of either sex. On six occasions when sweeps have been made in the swarm females have been caught on nights when no actual mating has been observed. Though it may be assumed that such females were in the swarm for mating, these occasions are not included in the above tabulation. A drizzle does not inhibit either swarming or mating.

Mating pairs do not straightway fall to the ground, though they tend to drop below the swarm as they fly off, invariably westward. This direction is down-wind of whatever light air movement there may be, it is also towards the brightest part of the sky and thus the easiest to notice. Pairs have not been seen to separate as they are lost to sight after a few feet of movement, and for the same reason the position of the two sexes during the act cannot be observed, but the "solid" appearance of the pair suggests that the position is closely apposed, and not "tail to tail". Of 115 females captured *in copula* when netted 75 (65 per cent.) had sperm in the spermatheca, which is considerably lower than the 95 per cent. fertilisation index found in the very numerous hungry females attracted to the outside of the insectary screen near the bait animal. As pointed out in Senior White (1951) copulation and ejaculation are successive acts, and some of the 115 females from the swarms may

* During the dry season other work is slack, hence more evenings were available for observations than in more busy months. The increase in the number of swarms recorded in April–August should not be taken as proving that there is more swarming during these months than during the rest of the year.

have been netted before ejaculation had occurred. Females taken in the swarms appear invariably to be unfed, contrary to Russell and Ramachandra Rao's (1942) findings with *A. culicifacies*. Blood feeding appears to follow fertilisation, but whether a nectar meal is sought as an immediate "pick-me-up" after the energy-expenditure of the mating flight and before seeking a blood meal, is still uncertain.

On two occasions freshly bred virgins were released into a male swarm. On the first occasion they were seen to fly straight into it and mate, on the second they did not do so. There is therefore some psychological difference in behaviour on different nights. Males have been observed to leave the swarms in pursuit of females flying in its vicinity, and we are under the impression that when there is much mating, the swarm does not thicken to the usual degree, perhaps because the males are "better" employed than in the presumably epigamic dance. On other occasions 24-48-hour old males have been released near a swarm; few of these joined it, the majority flew off down-wind. We have released gold-dusted males about 50 yards east of a swarm and subsequently net-swept it. No marked specimens were recovered. Males taken from a swarm, and therefore presumably sexually activated, have been caged with 4-day old virgins, but no mating occurred, a dozen of the latter dissected next day all had the spermatheca empty. A cage of males has been held at the correct hour over a drain known to be a swarming site, at between 5 and 6 ft. of height, and tapped to disturb the inmates. No swarm formation was induced. But as in over 50 per cent. of the swarms observed mating was seen to occur, and as swarming has been seen throughout the stretch of high *aquasalis* density from the seaface to ten miles inland, it is concluded that, in the case of *aquasalis*, fertilisation is closely connected with male swarming, even if it is not obligatorily connected with it.

Summary.

In Trinidad *A. aquasalis* swarms over open areas, mostly grass, and over drains and not over scrub vegetation.

Swarming commences at a light incidence not exceeding 5 foot-candles.

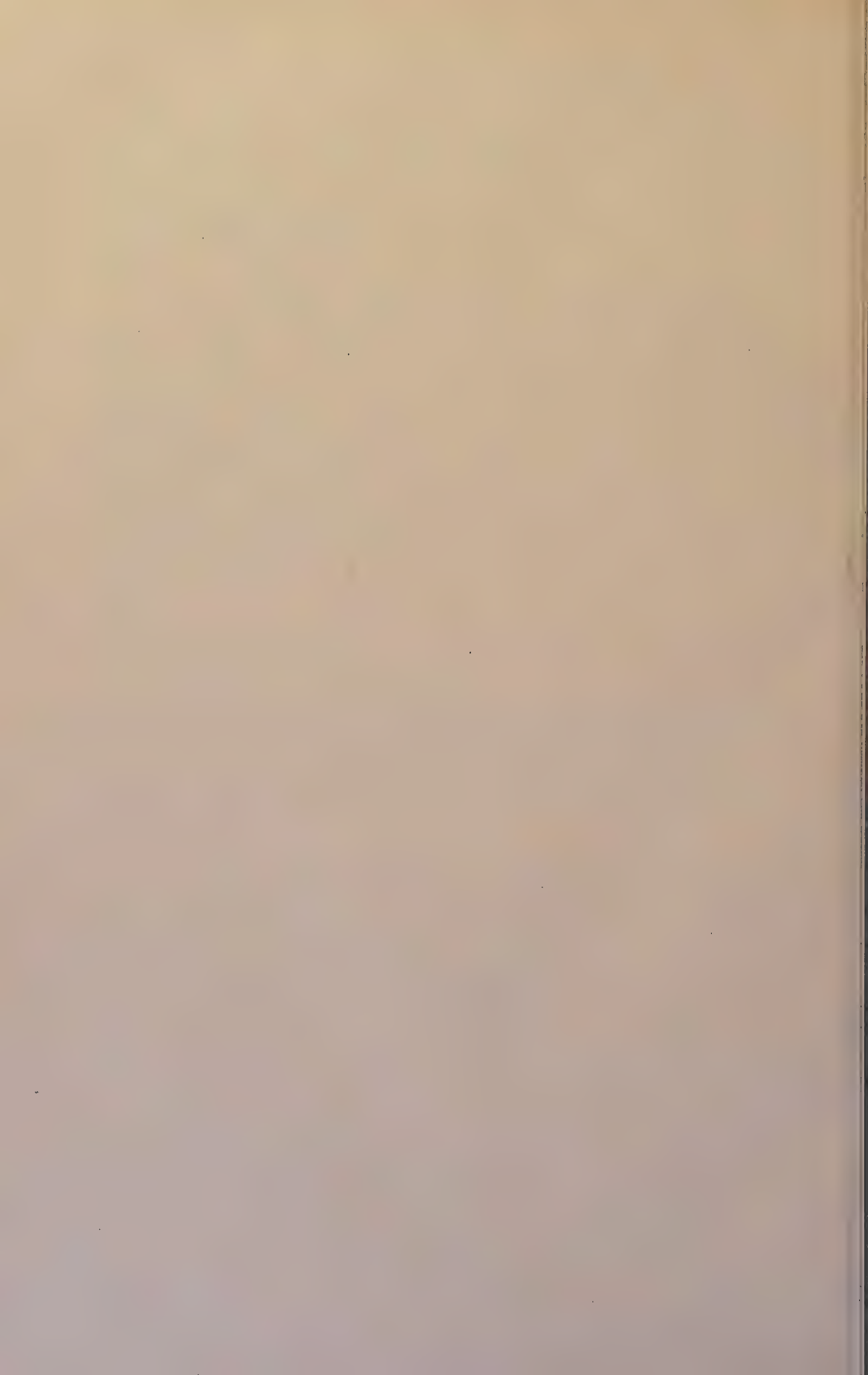
Mating has been observed in 54 per cent. of the swarms, the fertilisation index of females captured in them being 65 per cent.

The lag between the first sight of a swarm and the first observation of a mating pair averages 6.5 minutes, the light incidence at commencement of mating being no higher than an average of 0.8 foot-candles.

References.

- BATES, M. (1941). Laboratory observations on the sexual behavior of Anopheline mosquitoes.—J. exp. Zool., **86**, pp. 153-173.
- BELKIN, J. N., EHMANN, N. & HEID, G. (1951). Preliminary field observations on the behaviour of the adults of *Anopheles franciscanus* McCracken in Southern California.—Mosq. News, **11**, pp. 23-31.
- BORDAS, E. & DOWNS, W. G. (1951). Control of *Anopheles pseudopunctipennis* in Mexico with DDT residual sprays applied in buildings. Part IV. Activity pattern of adult *A. pseudopunctipennis* Theo.—Amer. J. Hyg., **53**, pp. 217-223.
- CAMBOURNAC, F. J. C. & HILL, R. B. (1940). Observation on the swarming of *Anopheles maculipennis*, var. *atroparvus*.—Amer. J. trop. Med., **20**, pp. 133-140.
- GEIGER, R. (1950). Climate near the ground. 2nd edn. (translation.) Cambridge, Mass., Harvard Press.
- HACKETT, L. W. & BATES, M. (1938). The laboratory for mosquito research in Albania.—Acta Conv. ter. trop. Malar. Morb., **2**, pp. 113-123.

- HARPER, J. A. (1944). Notes on the swarming of males of *A. funestus* (Giles), in East Africa.—E. Afr. med. J., **21**, pp. 150–151.
- MARSHALL, J. F. (1938). The British mosquitoes, p. 295. London, Brit. Mus. (Nat. Hist.).
- MICHELMORE, A. P. G. (1947). A popular misconception regarding humidity and the need for closer liaison between meteorologists and ecologists.—J. Ecol., **34**, pp. 107–110.
- NIELSEN, E. T. & GREVE, H. (1950). Studies on the swarming habits of mosquitos and other Nematocera.—Bull. ent. Res., **41**, pp. 227–258.
- RAMACHANDRA RAO, T. & RUSSELL, P. F. (1938). Some field observations on the swarming and pairing of mosquitoes, particularly *A. annularis*, in South India.—J. Malar. Inst. India, **1**, pp. 395–403.
- RUSSELL, P. F. & RAMACHANDRA RAO, T. (1942). On the swarming, mating and ovipositing behavior of *Anopheles culicifacies*.—Amer. J. trop. Med., **22**, pp. 417–427.
- SENIOR WHITE, R. A. (1951). Studies on the bionomics of *Anopheles aquasalis* Curry, 1932. Part II.—Indian J. Malar., **5**, pp. 465–512.
- THOMSON, R. C. MUIRHEAD. (1948). Studies on *Anopheles gambiae* and *A. melas* in and around Lagos.—Bull. ent. Res., **38**, pp. 527–558.
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THE TABANIDAE OF THE ANGLO-EGYPTIAN SUDAN.

By D. J. LEWIS, M.A.

Medical Entomologist, Stack Medical Research Laboratories, Khartoum.

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Between 1790 and 1900 many travellers wrote in general terms about TABANIDAE and their effect on man and animals in the Sudan. These accounts are being summarised by the author in a paper in the press and some of them provide a useful background for the modern study of these insects.

Since 1900 many species have been identified and their general distribution ascertained. The important direct effect of bites of Tabanids has long been known, and in the last 50 years studies on the distribution of tsetse flies and of animal trypanosomiasis have given an indication of the part that TABANIDAE probably play in the transmission of diseases of domestic animals.

This paper is chiefly an account of distribution, based on collections made by the writer and many others, and of the economic effect of the flies, on which there is much published information.

Some vernacular names of Tabanids have been recorded, by Balfour (1906), King (1908) and Lewis (1952, in press). The most suitable one for general use in the Sudan is the local Arabic word *surret* (singular *surreta*), of which "serut" is an incorrect version.

Seventy species are known to occur in the Sudan.

The direct effect of the bites of Tabanids is severe, and the flies are believed to transmit cattle and camel trypanosomiasis and human loiasis.

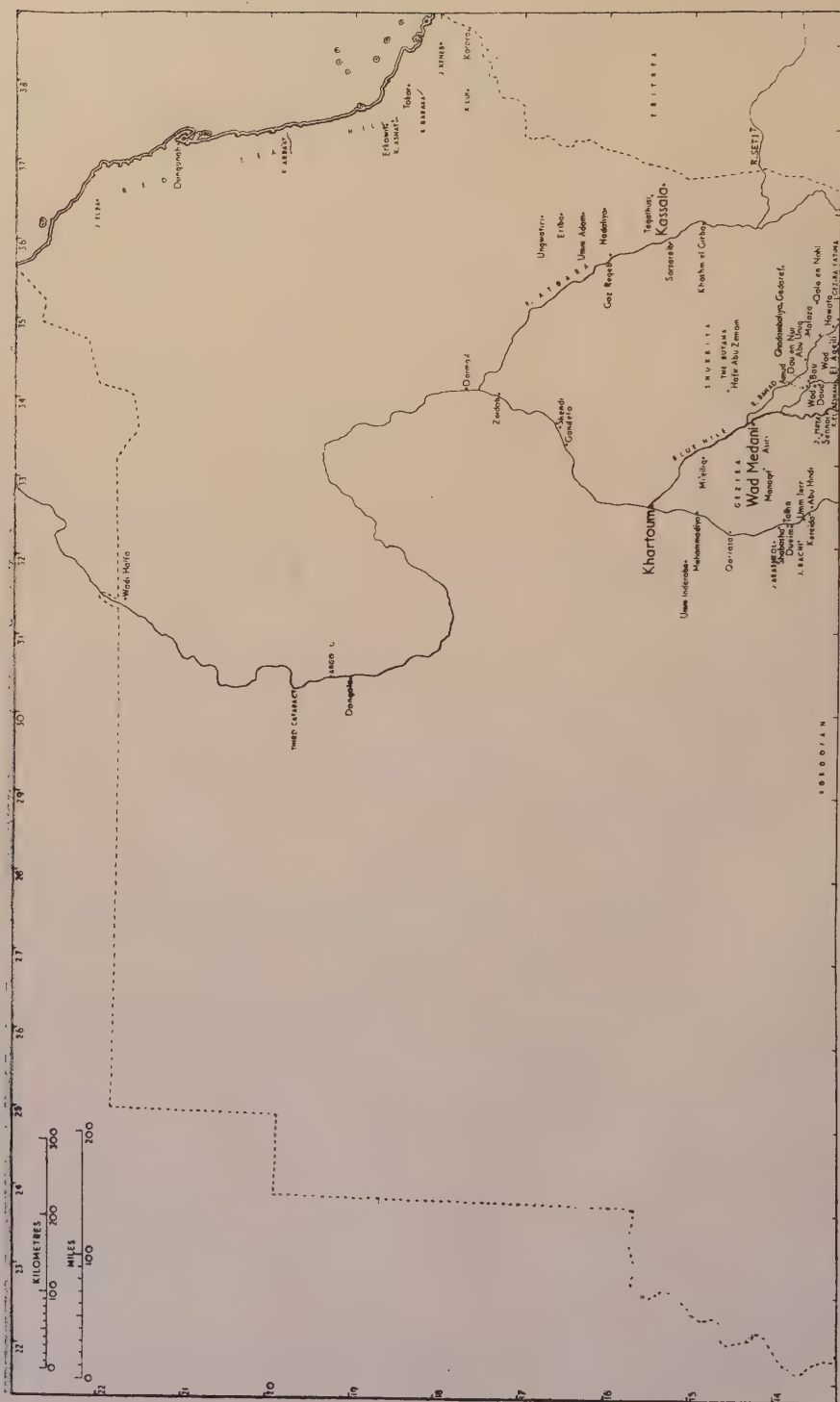


Fig. 1.—The northern Sudan showing places mentioned in the text.

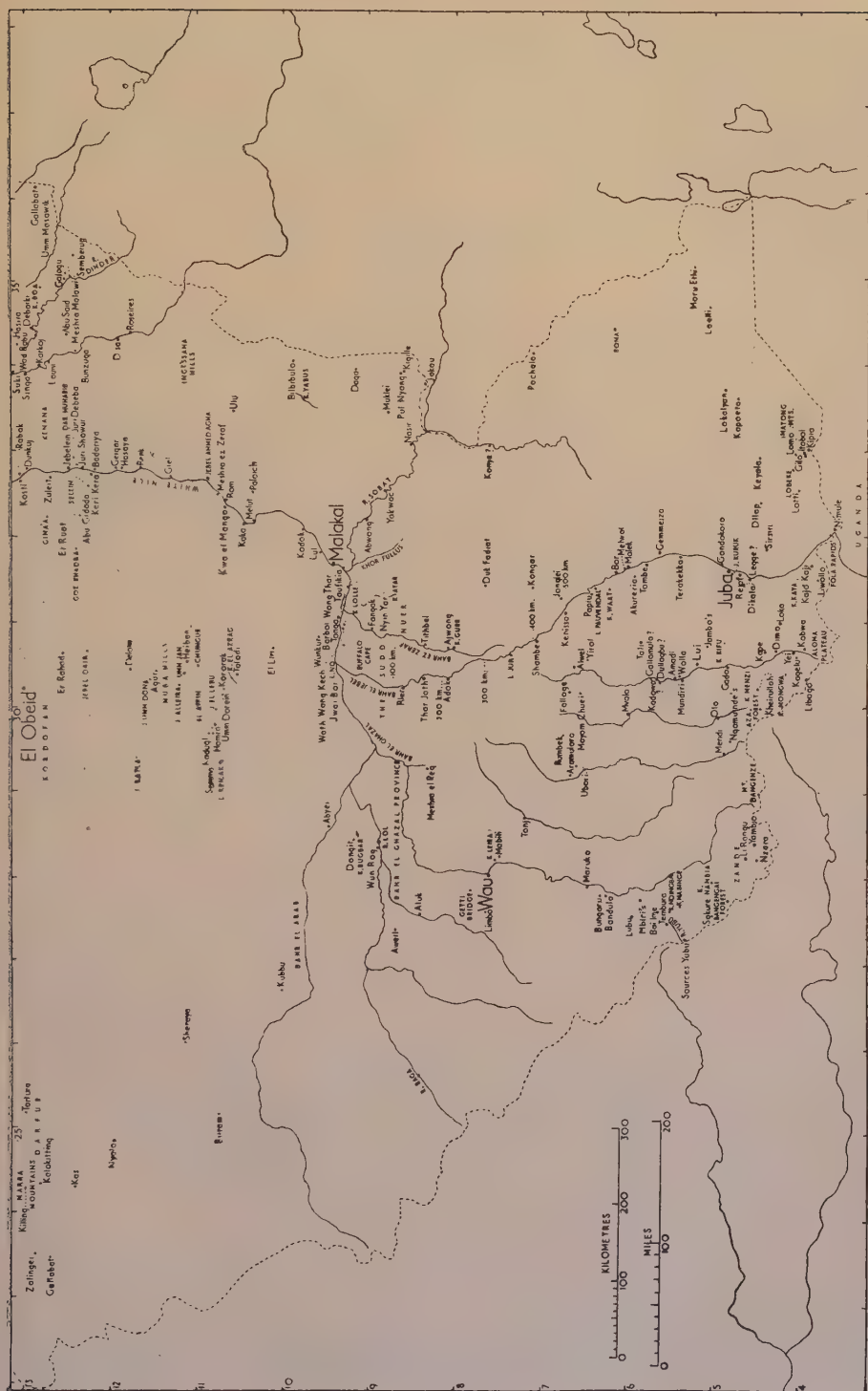


Fig. 2.—The southern Sudan showing places mentioned in the text.

Large numbers of people in many parts of the Sudan are nomadic or semi-nomadic pastoralists, partly owing to the low rainfall. In many cases, as Tothill and others (1948, p. 651) describe, their lives are closely bound up with those of their animals. Many of the people protect the animals by migrating out of the infested areas during the rainy season when the flies are prevalent. The flies thus exert a profound influence on the life of the country. They will cause additional problems as economic development entails settlement in areas which are at present evacuated during the Tabanid season.

This paper sets out the problem and it is hoped that it will facilitate experiments on control measures.

The Country.

The Sudan has been described by Tothill and others (1948) and many other writers. It comprises a large part of the Nile basin and contains a vast area of land which slopes gently from south to north (fig. 3). Rain is seasonal and decreases from south-west to north (fig. 4). In the extreme south-west, temperature and humidity are moderate but in the central Sudan conditions approach those of the northern deserts. Vegetation ranges from small areas of dense tropical rain forest to vast tracts of grassland, light bush and desert. The Nile passes successively through rapids, the great swamps of the Sudd region, which is bordered by a vast flood plain, a long quiet reach and, north of Khartoum, a series of rapids and quiet stretches. Its main tributary, the Blue Nile, has no flood plain apart from a series of narrow basins. Other notes on the country are given in later sections on areas.

Classification.

The species are arranged in this paper according to the advice of Mr. H. Oldroyd of the British Museum (Natural History) whose works on the Ethiopian genera other than *Hippocentrum* and *Haematopota* are in preparation. Many of the Sudan species can be identified from the existing publications of Austen (many papers), Bequaert (1930), Efflatoun (1930), Oldroyd (1952) and Surcouf and Ricardo (1909). Among papers dealing with general classification are those of Kröber (1927) on *Chrysops*, and Philip (1948). Austen (1906) and King (1908) published coloured illustrations of several species.

Descriptions of the early stages of several species are given by Efflatoun (1930) and Marchand (1920) and in papers by King which they quote.

Notes on the Species.

Synonyms are included which have been used in published records from the Sudan.

In the locality lists below "J" indicates jebel or hill, and "K" khor or watercourse. The known distribution is shown on maps (figs. 5-24) in which localities on the Nile are slightly displaced for clarity. There are some large areas, in which Tabanids have not been collected, which can be recognised by a glance at some maps of the commoner species.

***Tabanocella perpulcra* Austen (fig. 5).**

This species has been found at Sources Yubu.

***Pangonia magrettii* Bezzi (fig. 5).**

According to Austen (1908a, 1909) *P. magrettii*, which was described from Eritrean material, may be a dark form or subspecies of *P. rüppellii* Jaennicke which was first found in Ethiopia.

P. magretti has been recorded from Abu Gidada, Abu Hindi, Amud, Asir, Buram, J. Dair, Dau en Nur, Disa, Gedaref (King, 1911b) and 35 km. N.E. of it, Ghadam-baliya, Goz Khadra, Hafir Abu Zemain, Heiban, Jebelein (King, 1911b), Kadugli, Kaka, Kapoeta, Karora (King, 1911b), Kassala (Balfour, 1908b) and 32 km. S. of it in 1899 (Austen, 1909), J. Keneb area, Keyala, Khartoum, Kosti, J. Lebu, Loelli, Nyala, El Obeid (King, 1908), Qala en Nahal, Rufaa (Balfour, 1906; King, 1911b), Sarsareib, Suki and Talodi.

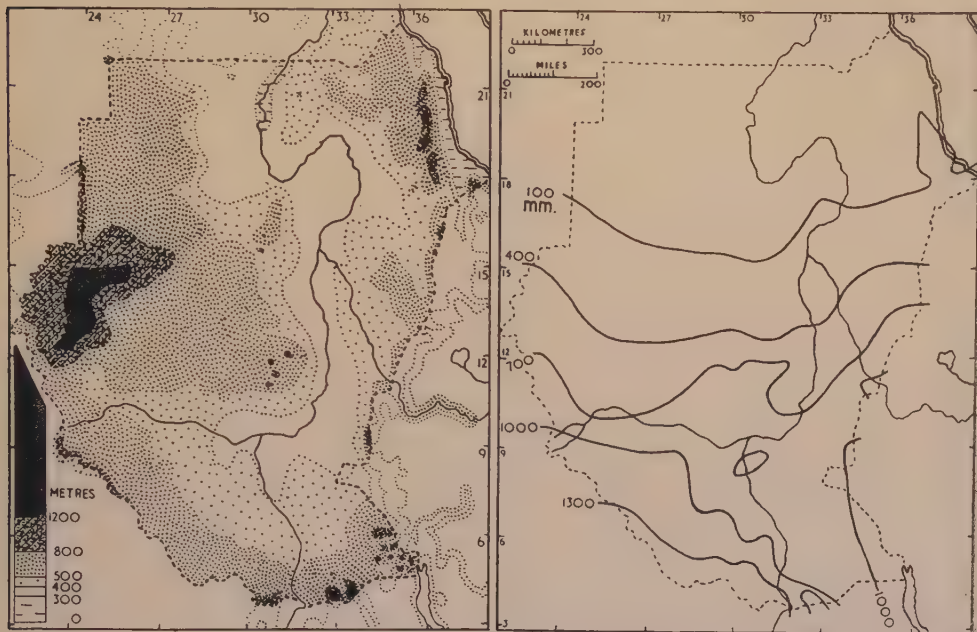


Fig. 3.—The Sudan, showing selected contours.

Fig. 4.—The Sudan, showing selected isohyets.

***Pangonia zonata* Walker (fig. 5).**

This species was taken on a camel at J. Keneb in April, 1926.

***Chrysops brucei* Austen.**

C. siccus Becker is a synonym, according to Bequaert (1930) and Kröber (1927).

Records are from Bor (King, 1911b), Falloge, Fola Rapids, Bahr el Jebel km. 120, 185, 230, 490 and 500, south of Kenissa, K. Lolle near Tonga (recorded by Becker, 1923, as *C. siccus*), Malek, Melwal, Nimule (Austen, 1909; Kröber, 1925), Shambe (King, 1911b) and N. of it, Thar Jath, Umm Jan and Wong Thar. Becker recorded this species from "Kordofan", and there is an unspecified record from north of Malakal.

***Chrysops distinctipennis* Austen (fig. 6).**

Records are from Barboi, Bor (King, 1911b), K. Bugbar, Fangak, Juai Bor, Kajo Kaji, Kape, Kheirullah, Kongor, K. Leira, Li Rangu (Bedford, 1936b, 1938), Mabili, Muklei, Lake No (King, 1911b), Talodi, Umm Dorein, Wong Thar and Yirol. Austen (1906) and Kröber (1927) recorded this species from the Sudan.

Chrysops funebris Austen (fig. 6).

Sources Yubu is the only known locality in the Sudan.

Chrysops longicornis Austen (fig. 7).

This species is known from Kalokitting, K. Leira, Li Rangu (Bedford, 1938) and Meridi.

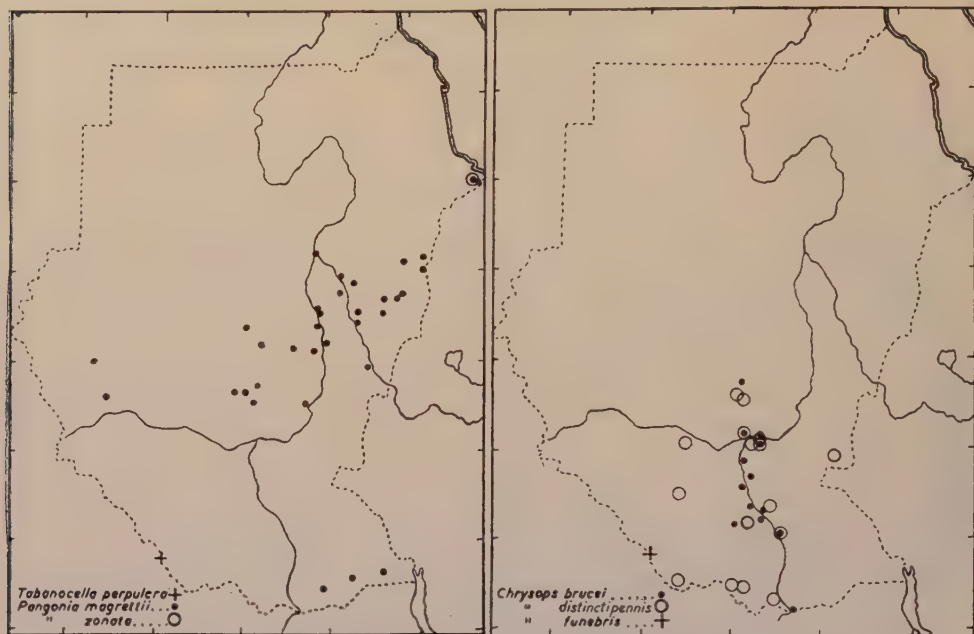


Fig. 5.—*Tabanocella perpulcra*, *Pangonia magretti* and *P. zonata*.

Fig. 6.—*Chrysops brucei*, *C. distinctipennis* and *C. funebris*.

Chrysops pusillula Austen (fig. 7).

There are records from K. Azraq, Heiban, Kororak, Mvolo, Talodi (Kröber, 1927), Umm Jan and Wau.

Chrysops silacea Austen (fig. 7).

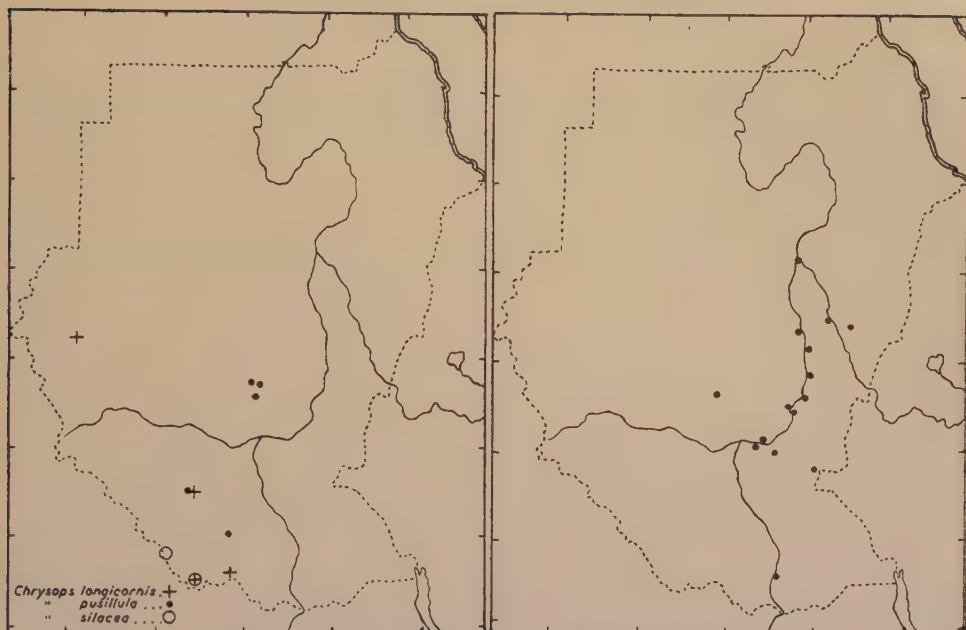
This species is known from Yambio, and from Sources Yubu (Woodman & Bokhari, 1941). There is an unconfirmed record from the Kheirullah area.

Ancala africana (Gray) (fig. 8).

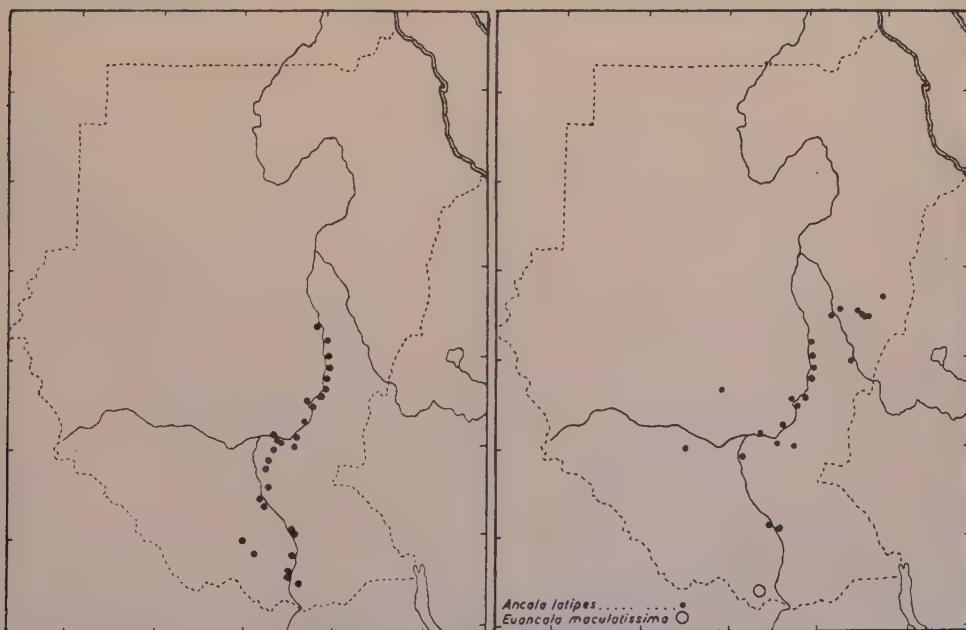
This species is known from J. Ahmed Agha, K. Fullus, Gondokoro, Jebelein (King, 1911b), Kadugli, Kaka, Khartoum, Kosti, Melut, Nasir (King, 1908), Nyin Yar, Renk, Sennar, Wad el Ageili and Wong Thar. Austen (1909) recorded it from the "White Nile, about 1862 (Consul Petherick)" and the Bahr el Ghazal, 1905.

Ancala fasciata var. *nilotica* (Austen) (fig. 9).

Records are from J. Ahmed Agha, Akureria, Amadi, K. Atar, Badariya, Barboi, Bor, K. Fullus, Giel, Jebelein (King, 1911b), Juba, L. Jur, Kaka, Kodok (Austen,

Fig. 7.—*Chrysops longicornis*, *C. pusillula* and *C. silacea*.Fig. 8.—*Ancala africana*.

1906, 1909 ; Surcouf & Ricardo, 1909), Kosti, Malakal, Malek, Melut, Mvolo, Nasir (King, 1908), Rejaf, Renk (King, 1926), Rom, Shambe, Sirsiri, Terakekka, Tithbel, Tonga, Wong Thar and Bahr ez Zeraf (9° and km. 95 and 116). Austen (1926) recorded it from the Nile.

Fig. 9.—*Ancala fasciata* var. *nilotica*.Fig. 10.—*Ancala latipes* and *Euancala maculatissima*.

***Ancala latipes* (Macq.) (fig. 10).**

Austen (1906, 1909) considered that *A. latipes* was the West African representative of *A. africana* and expected that the areas of the two species would be found to overlap in the Bahr el Ghazal or Kordofan. We now know, however, that the two species cover about the same area and that *A. latipes* is often more numerous on steamers or even occurs in the absence of *A. africana*.

Records are from Abwong, S. of J. Ahmed Agha, Badariya, Bau, Bor, Disa, Gezira Fatima, Khor Fullus, Gedaref, Giel, Hawata, Bahr el Jebel km. 120, Jebelein (King, 1911b), Juri Showur, Kadugli, Kaka, Lul, Mafaza, Melut, Papiu area, Renk, Sennar (in 1902, Austen, 1906), Tonga, Wad Daud and Wun Rog.

***Euancala maculatissima* (Macq.).**

This species is recorded from Loka.

***Atylotus agrestis* (Wied.) (fig. 11).**

Most Sudan records are under the name of *Tabanus ditaeniatus* Macq., which Efflatoun (1930) gives as a synonym.

The species has been reported from Abyei, Abu Gidada, Abu Said, Abu Uruq, Abwong, K. Ashat, Asir, Baa, Barboi (King, 1914), Bor, 11 km. N. of Eriba, Fangak, K. Fullus (King, 1914), near Gedaref (King, 1911b), Hadaliya, Hamra, Hasira, Hawata, Jebelein (King, 1911b), Juai Bor, Juba, Kadugli, Kaka, L. Keilak, Khartoum (King, 1908), Kodok, Kubbu, El Liri, Mafaza, Melut, Meshra ez Zeraf, Nyala, Mvolo, Paloich, Rejaf, Renk, Sennar, Sheraya, Singa, Suki, Talha, Talodi, Tokar, Umm Adam, Umm Darraqa, Ungwatiri, Wad Daud, Wad el Ageili, Wad Babu, Wad Medani, Wong Thar, Wunkur (King, 1911b) and Zeidab (King, 1910a, 1911). Austen (1906) recorded it from the Sudan.

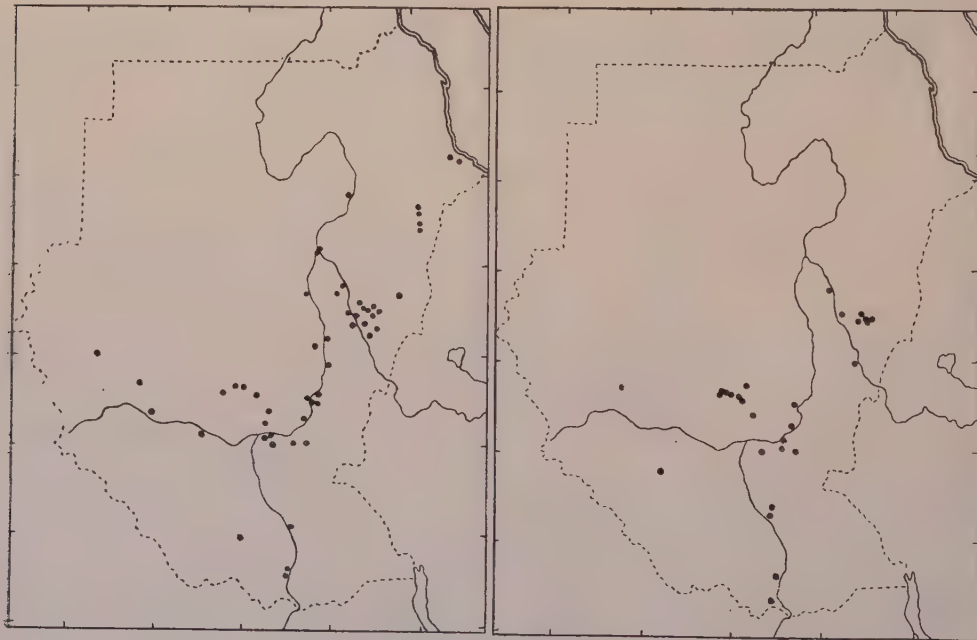


Fig. 11.—*Atylotus agrestis*.

Fig. 12.—*Atylotus fuscipes*.

***Atylotus fuscipes* (Ric.) (fig. 12).**

Records are from Abwong, Aluk, Bau, Disa, K. Fullus, Gezira Fatima, Hamra, Hawata, Heiban, Jonglei, Juba, Kadugli, Kaka, K. Kaya, Kodok, Kongor (atypical with some rather pale markings), Kororak, J. el Lebu, El Liri, Mafaza, Malakal, Semma, Sheraya, Talodi, Wad el Ageili, Wad Daud and Wad Medani.

***Tabanus besti* Surc. (fig. 13).**

This is known from Sources Yubu.

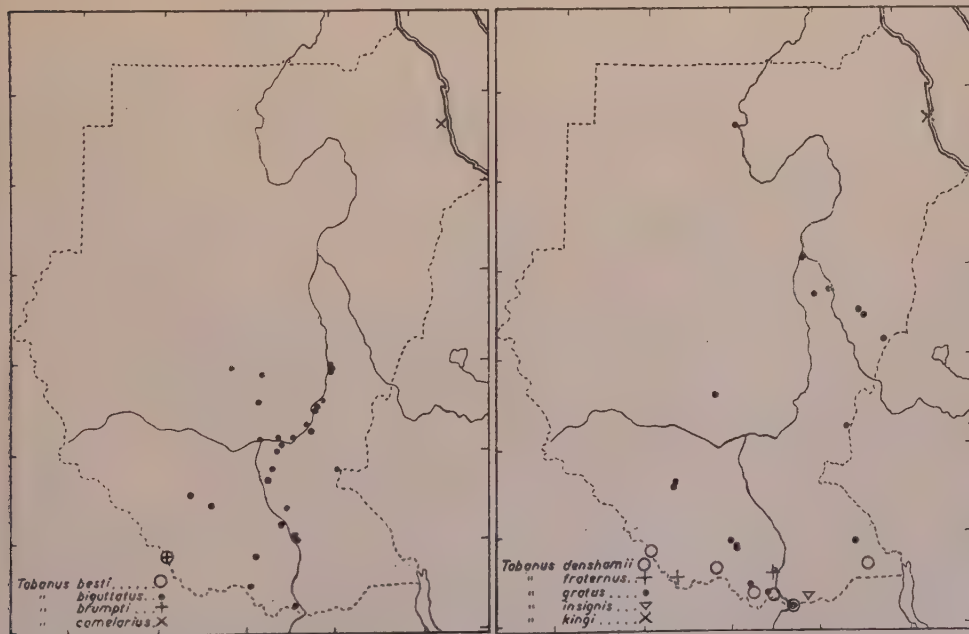
***Tabanus biguttatus* Wied. (fig. 13).**

This species is subject to colour variation (King, 1908).

It has been found at Agur, Ajwong, Amadi (on hippopotamus, E. T. M. Reid), Bor, Fangak, Hasaya, Jebelein (King, 1911b), Kajo Kaji, Kaka, J. Katla (King, 1911b), S. of Kenissa, Kheirullah area, Kodok (in 1900, Austen, 1906, 1909), Kongor (King, 1911b), Kwa el Mango, Malek (many in wrecked aeroplane (Mellor, 1932), Melut, Nasir (King, 1908), Nimule (Austen, 1909), Lake No, Renk, Rom, Talodi (King, 1911b), Taufikia (King, 1908), Tithbel, Tonga, Tonj, Wau, and Bahr ez Zeraf mouth. Austen (1909) recorded this species from 48 km. south of the Sobat, and from the White Nile at 11°N. Kröber (1939) quotes Wiedemann (1830) as stating that it occurred as far north as Egypt. It is not known in that country, however, and Wiedemann was probably referring to a specimen from the Sudan.

***Tabanus brumpti* Surc. (fig. 13).**

According to Austen (1909) this is a valid species very similar to *T. ruficrus*. It has been found at Sources Yubu.



Tabanus camelarius Austen (fig. 13).

Kröber considered that this species might be a synonym of *T. gratus*. It has been found at the K. Arbaat (Austen, 1911b).

Tabanus denshamii Austen (fig. 14).

Specimens from Loelli and Sources Yubu lack the lateral pale triangles. This species is known from Kajo Kaji, Loelli, Loka, Meridi, Nimule and Sources Yubu.

Tabanus fraternus Macq. (fig. 14).

This is known from the J. Kuruk area and Nzara.

Tabanus gratus Loew (fig. 14).

As in Egypt (Efflatoun, 1930) this species is widely distributed but uncommon in most areas. It has been found at Bilbibulo, Boma Plateau, Dimo, Dullagbu, Gallamula, Getti Bridge, Hawata, Juba, Kadowa, K. Kaya, Lake Keilak, Khartoum, Mafaza, Manaqil, Mvolo, Nimule (Austen, 1909 ; Surcouf & Ricardo, 1909), 5 km. E. of Semberug, Third Cataract (King, 1911b), Wad Medani and Wau.

Tabanus insignis Loew (fig. 14).

In the Sudan this species is only known from Lotti.

Tabanus kingi Austen (fig. 14).

King (1911a) took this species on a camel at the K. Arbaat.

Tabanus laverani Surc. (fig. 15).

This species has been found at Aweil, Mount Bangez, Kagelu, Li Rangu, Loka, about 15 km. N. of Ubori (found by F. C. Selous, the well-known hunter and collector of game animals, in some numbers, Austen, 1912a) and Yirol.

Tabanus leucostomus Loew (fig. 15).

This species occurs at Chungur, Kadugli and Karora (Austen, 1911b).

Tabanus mordax Austen (fig. 15).

Records are from K. Arbaat (Austen, 1911b), J. Elba (Kröber, 1929 ; Efflatoun, 1930), Erkowit, Karora (Austen, 1911b ; Efflatoun, 1930) and K. Lui.

Tabanus morsitans Ric. (fig. 15).

In the Sudan this species is only known from Kapoeta.

Tabanus nagamiensis Carter (fig. 15).

This species has been found at Gado, Li Rangu and Yirol.

Tabanus obscuripes Ric. (fig. 15).

This species is recorded from the Kheirullah area.

Tabanus par Wlk. (fig. 15).

In distinguishing this from the rather similar species, *T. obscuripes*, which is uncommon, and *T. thoracinus*, I am informed by Mr. H. Oldroyd that the apparent darkening of the tarsi and tips of the tibiae in *T. par* is produced by a covering of black hairs.

T. par has been recorded from Bor, Dongit area, K. Fullus (King, 1910a), Gemmeiza, K. Gurr, Hamra, Bahr el Jebel km. 55, 57, 60 and 170, Jebelein (King, 1910a), Juri Showur, Kadugli, Kaka, Kenissa and S. of it, Kodok, K. Leira, Melut, Mvolo, Nasir (King, 1908), Lake No, Pul Nyang, Renk, Rier area, Rom, Shambe, and S. of it, Taufikia, Thar Jath, Tonga and Wath Wang Kech and Wau. Neave (1906) recorded it from the Rumbek district.

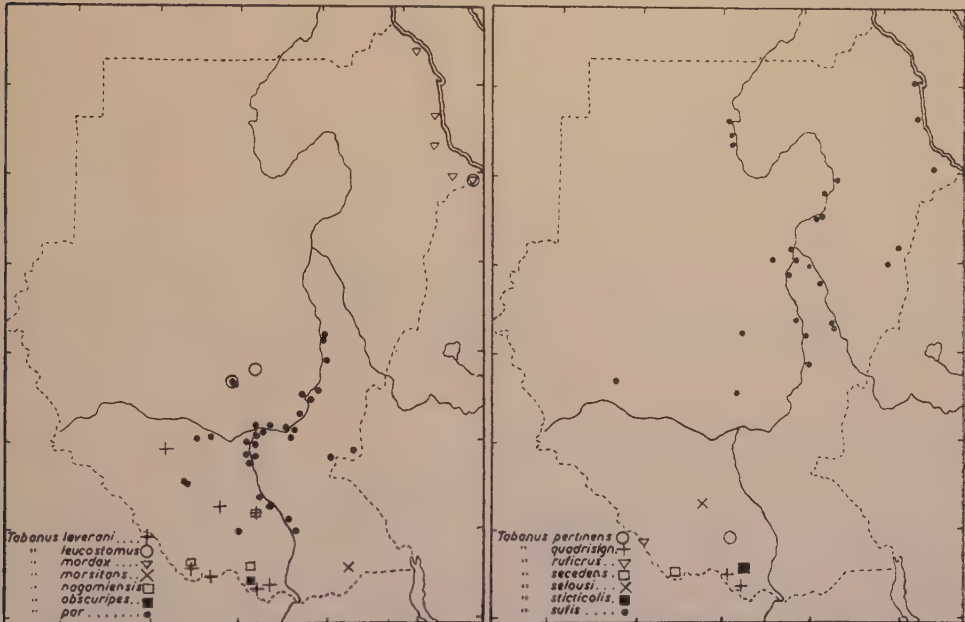


Fig. 15.—*T. laverani*, *T. leucostomus*, *T. mordax*, *T. morsitans*, *T. nagamiensis*, *T. obscuripes* and *T. par*.

Fig. 16.—*T. pertinens*, *T. quadrisignatus*, *T. ruficrus*, *T. secedens*, *T. selousi*, *T. sticticollis* and *T. suffis*.

Tabanus pertinens Austen (fig. 16).

Austen (1912b) recorded this species from the southern Sudan. It has been found at Kadowa.

Tabanus quadrisignatus Ric. (fig. 16).

This species occurs at Kagelu and Kheirullah.

Tabanus ruficrus Pal. de B. (fig. 16).

In the Sudan this species is only known from Tembura.

***Tabanus secedens* Wlk. (fig. 16).**

According to Austen (Rev. appl. Ent., (B) **10**, p. 110, 1922), Efflatoun (1930) and Kröber (1939) *T. secedens* is a synonym of *T. taeniola* P. de B., but in the present paper I follow the advice of Mr. Oldroyd and treat it as a species.

According to Bequaert (1930) it is strictly West African and Austen's (1909) records from Gondokoro and Nimule therefore seem doubtful. *T. secedens* is known to occur at Yambio.

***Tabanus selousi* Austen (fig. 16).**

The describer pointed out that this species resembled and was closely allied to *T. laverani* Surc. The type specimen was obtained about 15 km. north of Ubori on 18.iii.1911 by Selous.

***Tabanus sticticollis* Surc. (fig. 16).**

This species is recorded from Kape.

***Tabanus sufis* Jaen. (fig. 16).**

King (1911b) noted that specimens from K. Arbaat were pale.

T. sufis has been recorded from K. Arbaat (King, 1911b), Argo Island, K. Baraka, Darmali (King, 1911b), Dongola, Dungunab, Gandeto, Jebelain and Khartoum (King, 1911b), Khashm el Girba, Kosti, Launi, Mieiliq, Muhammadiya, Qarrasa, Rahad, Renk and Shendi (King, 1911b), Sheraya, Talodi, Tegallhusi, Third Cataract (King, 1911b), Umm Inderaba, Wad Medani and Zeidab. This species was also found 18 km. west of Asmara in Eritrea.

***Tabanus taeniola* Pal. de B. (fig. 17).**

T. dorsivitta Wlk., *T. pictipes* Becker, *T. socius* Wlk., *T. variatus* Wlk. and *T. virgatus* Aust. are synonyms (Austen, Rev. appl. Ent., (B) **10**, p. 110, 1922 ; Efflatoun, 1930 ; Kröber, 1939) under which *T. taeniola* has been recorded from the Sudan.

T. fraternus, *T. kingi*, *T. quadrisignatus*, *T. secedens* and *T. sticticollis* are not unlike it.

T. taeniola does not usually vary much in size, but a few small females with a wing length of 10 mm., instead of the usual 15 or so, were taken at K. Leira and Wad Medani.

The abdominal median dorsal pale markings vary from broad triangles to narrow stripes which together approximate to a straight line. In most specimens triangles are seen but some from Aramularo, Buffalo Cape, Juba, Lake Jur, Kapoeta, Keyala, K. Leira, Loelli, K. Nambia, Umm Jerr and Wau (mostly in the south) showed lines. There is considerable variation in a single locality, the Buffalo Cape specimen being taken with 19 others showing triangles.

T. taeniola has been recorded from Abu Gidada, Abu Said, Abu Uruq, Adok, El Affin, Alwel, Aramularo, K. el Atshan, Badariya, Barboi (King, 1914), K. Boa, Bor (King, 1910a), Buffalo Cape, Bunzuga, Daga area, Debarki, Delami, Dueim (King, 1911b), Duk Fadiat, Dunkuj, Fångak, Gezira Fatima, K. Fullus, Galegu, Giel, Hamra, Hawata, Heiban, Bahr el Jebel km. 120, Jebelain (King, 1911b) Juba, Juri Debeba, Juri Showur, Lake Jur, Juai Bor, Kaka (Lewis, 1952), Kadugli, Kapoeta, Karkoj, Lake Keilak, Kenissa (King, 1911b) and S. of it, Kereida, Keri Kera, Keyala, Khartoum (Balfour, 1906), Kigille area, Kodok (Austen, 1906 ; Balfour, 1904 ;

Theobald, 1904, as *T. dorsivitta*), Kongor, Kosti (Becker & others, 1923, as *T. pictipes*), Kubbu, Kwa el Mango, K. Leira, Loelli, Lui, Lul, Malakal, Malek, Melwal, Melut, Meshra Malawi, Meshra er Req, K. Nambia, Nasir (King, 1908; Wenyon, 1908), Lake No, Nyala, Paloich, Pochala, Pul Nyang, Rabak (Balfour, 1906), Renk and N. and S. of it, Sennar (in 1899, Austen, 1906), Shabasha, N. and S. of Shambe, Shendi, Sheraya, Singa, Tali, Talodi, Tartura, Terakekka and N. of it, Thar Jath, Tithbel, Tonga (Becker & others, 1922, as *T. pictipes*), Torit, Ulu area, Umm Darraqa, Umm Dona, Umm Inderaba, Umm Jerr, Umm Masawik, K. Waat, Wad el Ageili, Wad Daud, Wad Medani, Wau, Wong Thar, Wunkur, Yirol, Bahr el Zeraf km. 116 and at 9°N., and Zuleit. Austen (1926) recorded this species from the Nile. According to Efflatoun (1930) it occurs from the Mediterranean to Wadi Halfa. Specimens which may belong to this species have been obtained at Li Rangu and Yambio.

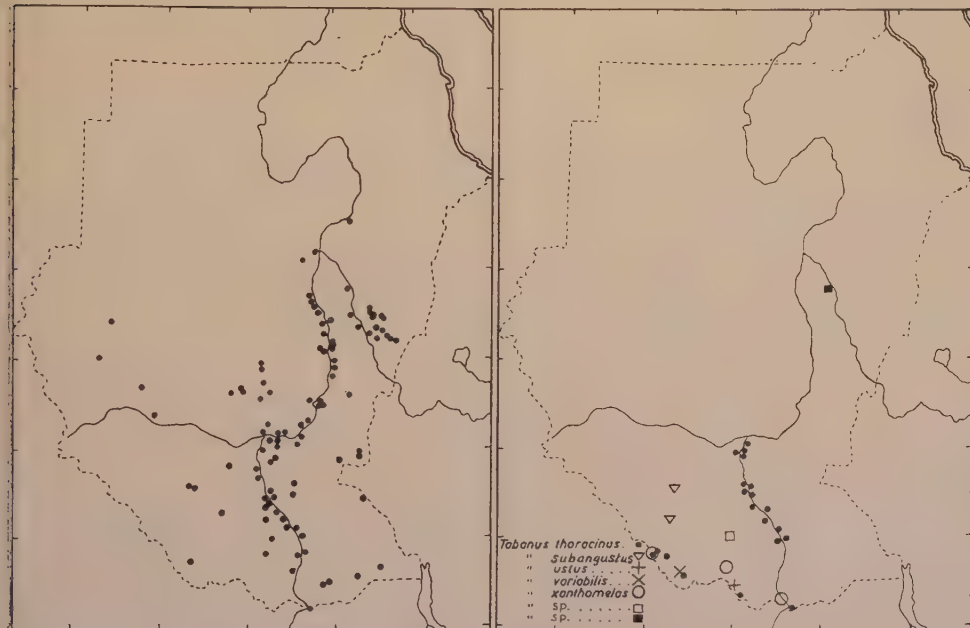


Fig. 17.—*T. taeniola*.

Fig. 18.—*T. thoracicus*, *T. subangustus*, *T. ustus*, *T. variabilis*, *T. xanthomelas* and two undetermined species.

***Tabanus thoracicus* Pal. de B. (fig. 18).**

This species has been recorded from Adok, Bor, Buffalo Cape, Bahr el Jebel km. 30, 35, 95, 120, 235, 285, 335, 490 and 500, S. of Kenissa, Li Rangu, Libogo, K. Mabinge, S. of Malek, Nimule (Austen, 1909), Lake Pauvendal, Rier, Shambe, Sources Yubu and Tembura.

***Tabanus subangustus* Ric. (fig. 18).**

This species has been recorded from Wau (King, 1908, 1911b; Ricardo, 1908; Surcouf & Ricardo, 1909) as *T. unitaeniatus* Ric.* It also occurs at Maruko.

* The specimen from Wau, recorded by King, is in the British Museum collection, and is not *unitaeniatus*, but *subangustus* Ricardo, described in the same paper. *T. subangustus* occurs in the Northern Territories of the Gold Coast, and in Northern and parts of Southern Nigeria, and probably extends across the intervening areas to the Sudan. Previously little has been known about this species, except that it was attracted to light. Recently Dr. Vanderplank sent me over 350 females which came to a light in one evening at Katabu, in N. Nigeria.—H. Oldroyd.

Tabanus ustus Wlk. (fig. 18).

In the Sudan this is only known from the K. Menzi.

Tabanus variabilis Loew (fig. 18).

This is known from Li Rangu.

Tabanus xanthomelas Austen (fig. 18).

This species is known from Kajo Kaji, Olo area and Sources Yubu. Austen (1912a) recorded it from the Sudan.

Tabanus sp. (fig. 18).

An undescribed species has been found at Mvolo.

Tabanus sp. (fig. 18).

Another undescribed species has been found at Wad Medani.

Haematopota abyssinica Surc. (fig. 19).

This species is known from Kajo Kaji, K. Kaya, Legge, Loka, Mvolo and R. Raga.

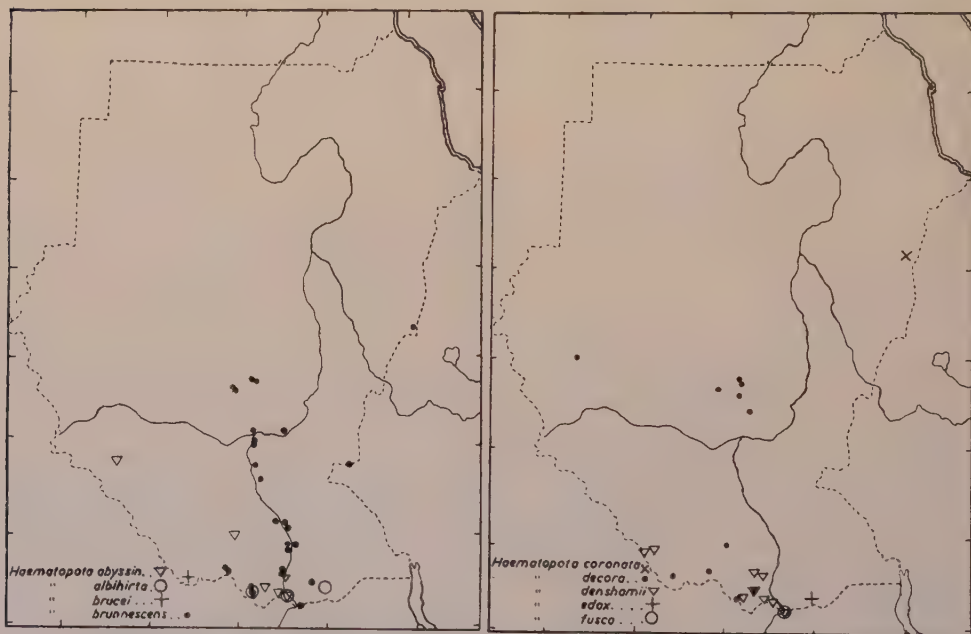


Fig. 19.—*Haematopota abyssinica*, *H. albihirta*, *H. brucei* and *H. brunnescens*.

Fig. 20.—*H. coronata*, *H. decora*, *H. denshamii*, *H. edax* and *H. fusca*.

Haematopota albihirta Karsch (fig. 19).

According to Mr. Oldroyd this is an earlier name for the species usually known as *mactans* Austen. It is known from Kajo Kaji, Lomo and Yei.

***Haematopota brucei* Austen (fig. 19).**

The only Sudan record is from Yambio.

***Haematopota brunnescens* Ric. (fig. 19).**

This species is known from El Affin, N. of Bor, Buffalo Cape, Dilap area, Gallabat (Kassala Province), Gemmeiza, Hamra, Heiban, Bahr el Jebel km. 45, Jokau, Juba, Kadugli, Kagelu, S. of Kenissa, Malek, Meridi, R. Mongwa, Ngamunde's, Nimule, Lake No, Rejaf, Taufikia (King, 1911b), Terakekka, Thar Jath, Tombe, Umm Jan and Bahr ez Zeraf 30 km. S. of K. Gurr.

***Haematopota coronata* Austen (fig. 20).**

The only Sudan record is from Tegalhusi.

***Haematopota decora* Wlk. (fig. 20).**

This species is known from Alleira, Amadi-Mvolo area, K. Azraq, Heiban, Kadugli, Kobwa, Li Rangu (Bedford, 1938), El Liri, Loka, Meridi and Nyala.

***Haematopota denshamii* Austen (fig. 20).**

This species is known from Bai Ime, W. of Juba, Kagelu, Kajo Kaji, K. Kifu, Liwollo, Loka, Nimule (on cattle, Austen, 1908a, 1909), Sources Yubu and Yambio.

***Haematopota edax* Austen (fig. 20).**

This species has only been found on the Imatong Mts.

***Haematopota fusca* Austen (fig. 20).**

The only Sudan record is from Nimule (on cattle, Austen, 1908b).

***Haematopota griseicoxa* Oldroyd (fig. 21).**

This species occurs at Sources Yubu.

***Haematopota harpax* Austen (fig. 21).**

This species occurs at Kagelu.

***Haematopota hirta* Ric. (fig. 21).**

This species occurs at Itabol.

***Haematopota lewisi* Oldroyd (fig. 21).**

This species is known from Bungaru, Jambo's, K. Kifu, Lubu, Maruko, Mvolo, K. Ndingba and Wolla.

***Haematopota nefanda* Edwards (fig. 22).**

This species is known from Sakure and Sources Yubu.

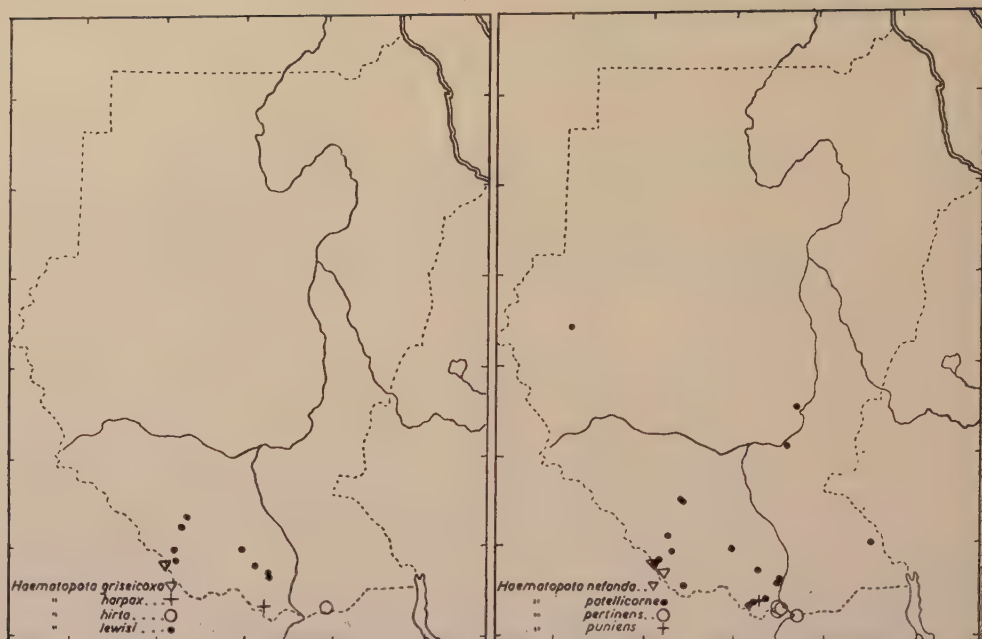


Fig. 21.—*H. griseicoxa*, *H. harpax*, *H. hirta* and *H. lewisi*.

Fig. 22.—*H. nefanda*, *H. patellicorne*, *H. pertinens* and *H. puniens*.

***Haematopota patellicorne* (End.) (fig. 22).**

I am informed by Mr. Oldroyd that specimens recorded from the Sudan as *H. pulchrithorax* Aust. (by Austen, 1906, 1909) and *H. vittata* Loew are *H. patellicorne*, although the two names are not synonyms of it.

H. angustipalpis (End.), which was described as a species of *Tylopelma*, and later placed in *Haematopota* by Bequaert (1930), is a synonym according to Oldroyd.

H. patellicorne is recorded from Bandula, Bungaru, Jambo's, Juba, Kajo Kaji, Kaka, Killing, Kobwa, K. Leira, Li Rangu (Bedford, 1938, as *H. vittata*), Libogo, Loka, Mabili, Malakal, Mbiri's, Mvolo, Nimule, Sources Yubu, Tembura and Yei.

***Haematopota pertinens* Austen (fig. 22).**

This species is known from Kajo Kaji and Nimule, K. Kaya and Liwollo.

***Haematopota puniens* Austen (fig. 22).**

This species occurs at Loka.

***Haematopota taciturna* Austen (fig. 23).**

Known localities are El Affin, Barboi, Delami, Disa, Hamra, Heiban, Kadugli, Kajo Kaji, Kodok, Loka, Lokalyan, Roseires and Talodi.

Lewis (1952) and Oldroyd (1952 p. 118) have referred to Marno's record of *Haematopota* sp. from the Blue Nile area. Mr. Oldroyd informs me that he considers it to be *H. taciturna*.

Haematopota tenuis Austen (fig. 23).

Known localities are Bor (King, 1911b), Bangengai Forest, Dikala and 15 km. N., Gallabat (Kassala Province), Jambo's, Kajo Kaji, K. Kaya, Legge, Limbo. Mayom Chuei, Mvolo, Shambe (King, 1911b), Talodi, Umm Dona and Wau.

Haematopota transiens Oldroyd (fig. 23).

Known localities are Jambo's, Li Rangu, Meridi and Sources Yubu.

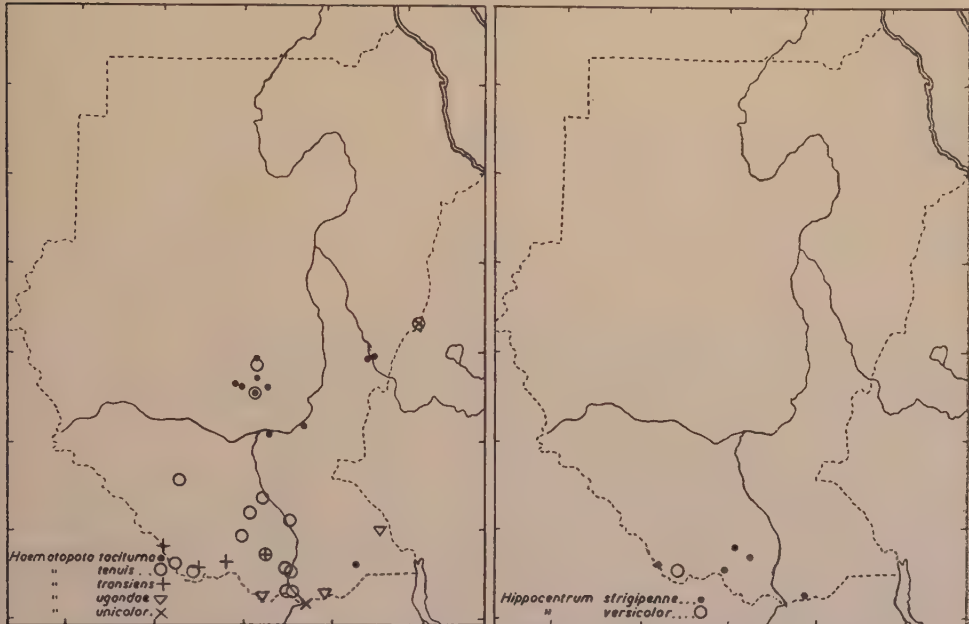


Fig. 23.—*H. taciturnae*, *H. tenuis*, *H. transiens*, *H. ugandae* and *H. unicolor*.

Fig. 24.—*Hippocentrum strigipenne* and *H. versicolor*.

Haematopota ugandae Ric. (fig. 23).

Known localities are Aloma Plateau, Boma Plateau, Gilo and Kipia area.

Haematopota unicolor Ric. (fig. 23).

Known localities are Amadi-Mvolo area, Gallabat and Nimule.

Hippocentrum strigipenne (Karsch) (fig. 24).

Known localities are Aza Forest, Bangengai Forest, Jambo's, Lotti and Mundiri.

Hippocentrum versicolor Austen (fig. 24).

This species is known from Li Rangu. King (1908) reported it from the Sudan.

Records that require further study or are erroneous.

A specimen of *Chrysops fuscipennis* Ric. is labelled Lake No, 1913, but as this species has not been recorded since then its presence requires confirmation.

A published record of *T. crocodilinus* Aust. from the Sudan is omitted because the locality, Dufile, is now in Uganda.

T. subfasciatus Becker, 1923, has not been reported since the describer recorded it from Sennar.

Distribution in General.

Most of the Sudan is part of the Sudanese zoogeographical Province of the Ethiopian Region. Certain natural faunal areas were selected by Lewis (1947, 1948) for describing the faunas of mosquitos and Simuliids. These have been altered for use in the present paper (fig. 25), after taking into account certain features of topography, geology, rainfall and vegetation, and the distribution of Cimicids, some mosquitos, *Phlebotomus* and Tabanids. Much information on vegetation is provided by Tothill and others (1948) and Morrison and others (1948). Some names and boundaries have been altered on the map and new divisions made. The features of each area are briefly described later.

The composition of the fauna.

Nearly all the Tabanids of the Sudan are Ethiopian species and most of them are widely distributed in that zoogeographical Region.

C. funebris, *C. silacea*, *T. besti*, *Hippocentrum strigipenne* and several other species occur mainly in the West African Sub-region and, in the Sudan, are more or less confined to the south-west. *C. silacea* probably reaches its eastern limit in the Sudan ; in Uganda and the eastern Congo it is replaced by *C. centurionis* Aust. (E. Afr. High Comm., 1951, p. 27).

A few montane species occur in the Imatong Mountains.

Pangonia zonata and *Haematopota coronata* are species of the Somali Arid District.

Five species are common to Egypt and the Sudan, *Atylotus agrestis*, *Tabanus gratus*, *T. kingi*, *T. sufis* and *T. taeniola* (Eflatoun, 1930 ; Kröber, 1939). *A. agrestis* also occurs in southern Europe and Arabia, and *T. sufis* in Palestine as well as the north of the Ethiopian Region.

Africa is particularly rich in species of *Haematopota* (Edwards & others, 1939) and many are found in the Sudan.

There are several examples of discontinuous distribution which are probably a relic of more extensive distribution in former times when rainfall was heavier. Two species in the Marra Mountains are widely separated from their other localities, and *T. leucostomus* in the Red Sea Hills is far from its other locality, in the Nuba Hills. There appears to be a considerable gap in the distribution of *T. taeniola* between Egypt and the Sudan.

Types of habitat.

The Sudanese zoogeographical Province is divided into the Savanna and Arid Districts and most of the Tabanids occur in the former.

There are enough locality records for several species to give some information on the type of country which they normally inhabit.

Dense forest : *Tabanocella perpulcra* ; *Chrysops funebris*, *C. silacea* ; *Tabanus besti*, *T. brumpti*, *T. ruficrus* ; *Haematopota griseicoxa*, *H. nefanda* ; *Hippocentrum strigipenne*.

High mountains : *T. insignis* ; *Haematopota hirta*, *H. ugandae*.

Riverain : *C. brucei* ; *Ancala africana*, *A. fasciata* var. *nilotica*, *A. latipes* ; *T. biguttatus*, *T. par*, *T. thoracinus* ; the two last evidently breed in permanent

swamps ; they are common in the papyrus region between Shambe and Lake No, whereas other riverain species tend to flourish where the swamps are seasonal.

Sloping country with seasonal rain and mainly temporary streams : *C. distinctipennis*, *C. pusillula* ; *Haematopota abyssinica*, *H. decora*, *H. lewisi*, *H. patelllicorne*, *H. taciturna*.

Flat country with seasonal swamps : *Pangonia magretti*.

The Red Sea Hills, mainly barren country with a few small perennial streams : *P. zonata* ; *T. camelarius*, *T. kingi*, *T. leucostomus*, *T. mordax*.

Species widely distributed in different types of country : *Atylotus agrestis*, *A. fuscipes* ; *T. gratus*, *T. sufis*, *T. taeniola* ; *Haematopota brunnescens*, *H. tenuis*.

Distribution in relation to classification.

Generally speaking the species of each genus tend to have the same type of distribution. *Tabanocella*, *Chrysops*, *Haematopota* and *Hippocentrum* are almost confined to the southern Sudan and the two central hilly areas which may be looked on as " outliers " of the south. The three species of *Ancala* are riverain. *Tabanus* includes several riverain species. *Tabanus*, *Pangonia* and *Atylotus* include the species which can exist in the relatively dry central Sudan.

As in several other families of insects there is a marked tendency for pale species to occur in arid country. Most species of the southern genera, *Chrysops*, *Haematopota* and *Hippocentrum*, are darker than most species of *Tabanus*. The very dark *T. xanthomelas* is one of the southern species which contrast with the pale *P. magretti*, *T. sufis* and other northern species. There are also pale subspecific forms. *Ancala fasciata* var. *nilotica* is a pale form which is perhaps restricted to the Sudanese savanna country (Bequaert, 1930). King (1911b) noted that *T. sufis* from the Khor Arbaat was paler than specimens from other areas. In *T. taeniola* the specimens with a narrow median stripe are mainly from the south. Pale specimens of *T. biguttatus* occur but it is doubtful if they have any geographical significance (Austen, 1906, 1909 ; Bequaert, 1930 ; King, 1908).

The areas in which Tabanids cause serious harm to domestic animals by biting.

In the south-west, Tabanids, many of them *Haematopota*, are not generally very annoying. Much of the area is tsetse country (fig. 27) so there are few cattle, and, perhaps partly for this reason, there are few complaints of Tabanids.

In the central Sudan, as far north as about 12°, riverain land is infested with Tabanids but it is not known how numerous they are inland.

Further north is flat clay country with scattered seasonal swamps and areas of light woodland. *T. taeniola* and *P. magretti* become abundant in the rains, the latter species usually extending further north than the former. The northern limit of this area of infestation is indefinite and varies somewhat from one year to another according to rainfall. It is approximately indicated in fig. 26 and corresponds roughly to the 500 mm. isohyet. It also corresponds in many places with the junction between clay and sand, but, where clay country extends far to the north, as in the Gezira, low rainfall seems to determine the northern limit of the " fly ". In some places tabanid-infested country with fly-free sandy patches merges gradually into fly-free sandy country with infested patches of clay. Curiously enough the northern limit does not extend northward along the rivers and *P. magretti* is troublesome inland from riverain areas where Tabanids are scarcely noticeable.

There is a small area around Karora where for a short time *P. magretti* makes it difficult to keep camels and cattle.

Seasonal Prevalence.

Tabanids occur throughout the year along the Nile between Shambe and Jebelein, on the R. Setit and in some other riverain areas. Inland they occur over vast areas during the rainy season, from July to September, and in some places they persist till later. They are more abundant in years of heavy rain. Near Karora Tabanids are common from February to April after the coastal winter rains.

Breeding Habits.

The breeding habits of several species which occur in the Sudan have been described by Gordon and others (1948), and by Marchand (1920) who quotes the numerous observations made by King in the Sudan. The present writer saw *T. gratus* laying eggs on grass about 8 cm. above the surface of a fish pond at Wad Medani in May at 5.10 p.m. Larvae and pupae of the same species were found among moss in a brick-lined irrigation channel in the same area in April, and the pupal stage was found to last eight days.

With regard to the annual outbreaks of Tabanids during the short rainy season of the central Sudan, the length of the breeding cycle and the method of aestivation are important subjects requiring further study. According to Marchand many Tabanids breed slowly and may have only one generation a year. Some species probably live for several months as larvae, hibernate or aestivate in this stage and later pass through a comparatively short pupal stage. The breeding habits of *P. magretti* are unknown. *Ancala fuscipes* is thought to hibernate as a larva (Neave, 1915). The larvae of *T. taeniola* were found by King to live for about two months or more in water. According to Surcouf (1924) some Tabanids can breed rapidly.

Habits of Adults.

Haunts.

According to numerous reports from the central Sudan, *P. magretti* and some other species are more or less restricted to patches of bush, and sometimes harbour in long grass. Camel and cattle men are well aware of infested bush and also of fly-free areas in the form of naturally open land and clearings near big villages.

At Wad Medani *T. gratus* and *T. taeniola* are often seen resting on tree trunks, and at Juba a male *Haematopota* was found in a leaf axil.

Attraction to inanimate objects.

It is known that Tabanids are often attracted to non-living objects. In North American (Cameron, 1926 ; Philip, 1931 ; Twinn & others, 1948), they are attracted to moving trains and to dwellings and stationary cars. In one instance flies settled on a car rather than a person, possibly attracted by the warmth of the former.

In the Sudan *P. magretti* and *Atylotus agrestis* sometimes follow trains without attacking the passengers, and *T. taeniola* and other species frequently board moving steamers. *P. magretti* often settles on cars, and Mr. G. H. Bacon has told me of an occasion when this species stopped attacking him and settled instead on a groundsheet which he had hung up in a tree to give shade. *T. biguttatus*, as mentioned above, has been seen to assemble around a wrecked aeroplane.

Tabanids are often seen to probe parts of the objects on which they settle, such as cars. On steamers *Ancala latipes*, *T. taeniola* and *T. thoracinus* probe canvas and woodwork, often where it is painted dark, and *P. magretti* has been seen probing the iron part of a concrete culvert and making no attempt to attack bystanders.

Powers of flight.

Some Tabanids have considerable flying powers ; Wigglesworth (1950) quotes an estimated speed of 31 miles an hour for one species. According to Austen (1909, 1920) they sometimes follow moving game animals and cars. In the Sudan *P. magrettii* and *Atylotus agrestis* readily fly alongside trains, apparently for several miles, and King (1910a) found that *T. taeniola* could follow cattle and game for great distances.

Transport by artificial means.

Whitfield (1939) found 28 *T. taeniola*, 4 *Ancala fasciata* var. *nilotica*, 1 *C. distinctipennis*, 1 *C. longicornis* and 1 *H. taciturna* in aircraft arriving at Khartoum from the south. One *T. kingsleyi* Ric., a West African species, was found in an aircraft landing at Khartoum.

Tabanids travel considerable distances in Nile steamers. At Juba the flies are said to come ashore from passing steamers and attack dairy cattle.

*Attraction to light.**

The males of several species, including *Ancala fasciata* var. *nilotica*, *T. par*, *T. suffis* and *T. taeniola*, are sometimes found at light, and occasionally also the females of *H. brunnescens*, *H. lewisi* and others.

Feeding habits.

There are many records of various species of Tabanids biting camels, cattle, donkeys, horses and man in the Sudan, and some species have been found on dogs (*T. gratus*), mules, wild buffalo (*Atylotus agrestis* and *T. taeniola*), Jackson's hartebeest (*T. par*), hippopotamus (*T. biguttatus*), oribi (*H. taciturna*) and waterbuck (*C. distinctipennis* and *T. sticticollis*).

It seems probable that most Tabanids will attack most large mammals but will attack man less readily.

It is a matter of experience that some species attack man vigorously in certain areas and not in others, and on some occasions and not others. Efflatoun (1930) stated that he had never been bitten by a Tabanid in Egypt. At Wad Medani *T. gratus* and *T. taeniola* are not uncommon but very seldom bite man. In the White Nile steamers *T. taeniola* sometimes bites fiercely and sometimes merely probes the woodwork. *P. magrettii* is sometimes very annoying and sometimes bites little. Carpenter (1925) found that an East African species bit much more readily on some days than others. Several species bite mainly during the heat of the day.

P. magrettii has been seen probing the dewlaps of cattle and is said to bite the bellies of camels. Like several other species of this genus (Tetley, 1918 ; Carpenter, 1925), it probes when flying.

King (1910a) noticed males of *T. par* feeding on flowers, and the present writer saw a female *T. taeniola* on mango flowers at Tali.

Parasites and Predators.

Trypanosomes and *Loa loa* are mentioned below. Wenyon (1908) reported finding *Herpetomonas* in *T. taeniola* and six other species. *Telenomus benefactor* and *T. kingi* (Hymenoptera, Proctotrypoidea, SCALIONIDAE), were obtained from the eggs of *Tabanus taeniola* at Jebelain and of *T. kingi* at the K. Arbaat, respectively, and were described by Crawford (1911). They are mentioned by Efflatoun (1930), King (1911a), Marchand (1920, pp. 116, 170 and 186) and Nikol'skaya (1948). A pseudo-scorpion has been found on *T. taeniola*.

* See footnote, p. 187.

No predators have been observed, but it is interesting to notice that much of the worst Tabanid area is without permanent water and presumably lacks many predatory fish and insects.

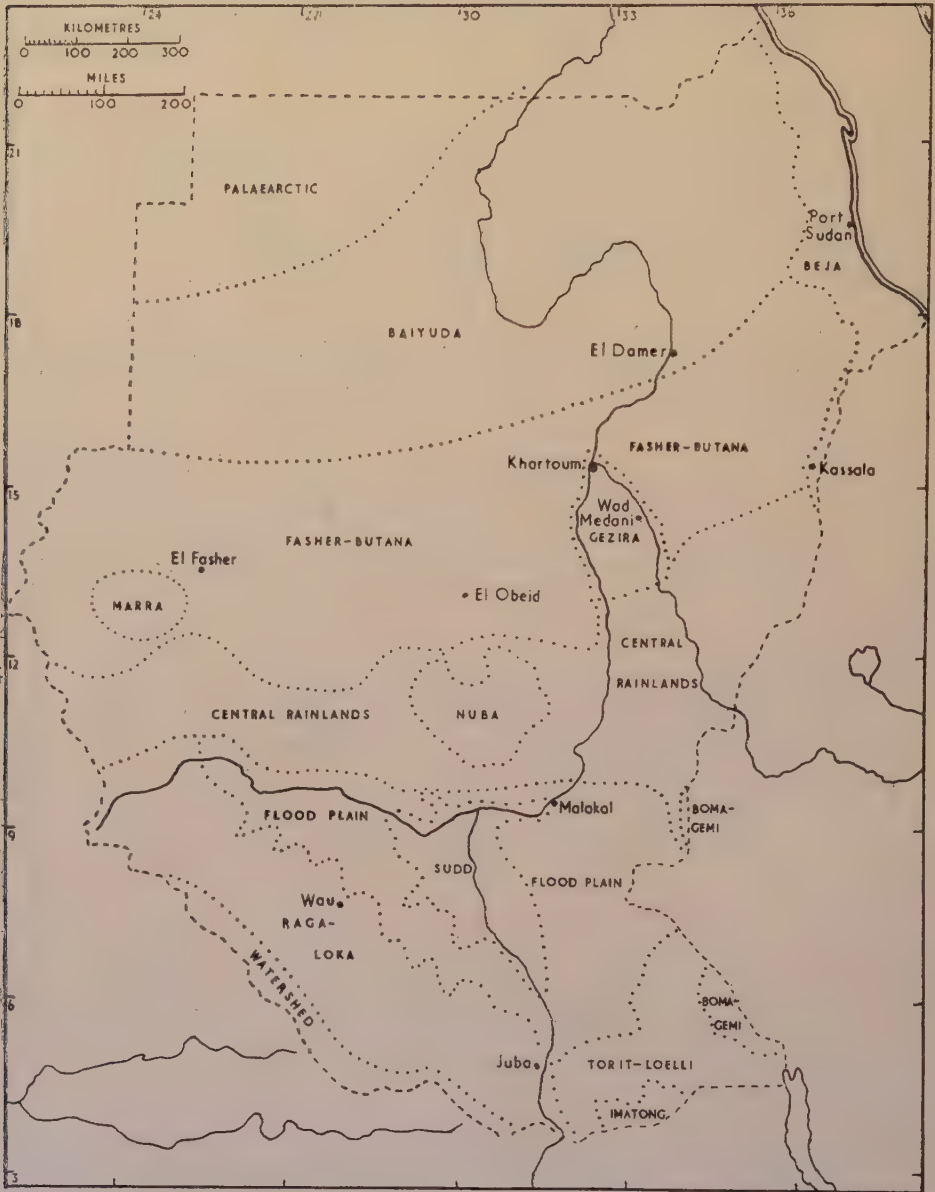


Fig. 25.—Faunal areas selected for discussing Tabanids.

Economic Importance.

The importance of Tabanids has been discussed by Matheson (1950) and by Zumpt (1949) who gives references to the principal papers on the subject. In addition to the harm caused by their bites Tabanids can transmit trypanosomiasis, human loiasis,

anthrax, haemorrhagic septicaemia (of buffaloes in the Far East), anaplasmosis of cattle and several other diseases. These extremely annoying flies are often disturbed while feeding and attack another host immediately. Thus they can transmit acyclically certain trypanosomes and other parasites which are present in the peripheral blood and can resist short exposures to the air. Many writers have discussed the transmission of cattle trypanosomes by Tabanids in Africa. Several species can transmit *Trypanosoma evansi*, an important parasite of camels in the Sudan, and *Tabanus thoracinus* can transmit *Trypanosoma congolense*. It appears that cattle trypanosomes are less suited than *T. evansi* for mechanical transmission, but that where many cattle stand close together Tabanids and possibly other flies may be important vectors of cattle trypanosomiasis.

The effect of bites.

When many Tabanids bite domestic animals, they can cause much irritation and loss of condition (Balfour, 1906) and a reduction in milk yield. Among the cattle of the Dinka and Nuer (Sudan Vet. Ser., Report for 1942, p. 11) "irritation produced by biting flies, apart from any disease that may be transmitted, is a more serious menace than is generally appreciated". According to Balfour (1906) animals can die from the effect of numerous bites.

There are two main types of cattle in the Sudan (Tothill & others, 1948, pp. 19, 21, 634, 635) which are not equally resistant to the effect of flies (Sudan Vet. Serv., Reports for 1930, 1934, 1941 and 1943). These are the long-horned small-humped cattle of the Flood Plain and Sudd Areas and the short-horned large-humped cattle of the nomadic Arabs, north of about 10°, which were brought to the country much later. The long-horned cattle can live in the south where many short-horned animals would die, a fact which has been partly attributed to flies which have worried imported cattle much more than local ones. Furthermore, southern cattle, and their owners, can endure the smoke from fires used for their protection.

The small Nuba cattle are little affected by the bites of flies (Colvin, 1939).

A single bite from *T. taeniola* is painful to man and causes considerable irritation, swelling and discomfort. If cattle are similarly affected the result of mass biting must be severe.

Camels are much more troubled by biting flies because their short-haired tails give little protection and they cannot twitch their skin much (Leese, 1927). Camels attacked by Tabanids roll frantically on the ground with their loads on.

It is uncertain to what extent Tabanids (gad-flies) cause animals to gad, or rush madly about. Austen (1939) pointed out that it is often difficult to find out which species of fly is responsible and suggested that *Hypoderma* might be a cause. King (1911b) recorded *Gasterophilus flavipes* (Oliv.) stampeding donkeys at Renk, although they took little notice of Tabanids, and the present writer has seen donkeys similarly affected by the same species at Dueim. King (1910a) reports that animals were driven frantic by *T. taeniola* on river barges, and others have described the apparent effect of Tabanids in exciting animals. In one instance a camel, attacked by *P. magretti*, rolled in the smoke fire made to protect it and was badly burned. It is probable that the larger Tabanids do excite some animals, particularly camels.

Trypanosomiasis of cattle.

The tsetse-fly area contains comparatively few cattle but is important as a focus from which the disease can spread. The known northern limit and outliers of *Glossina* (after Lewis, 1949) are shown in fig. 27, and the Sudan Veterinary Service is at present conducting an extensive survey to ascertain if any other areas are infested.

The situation beyond the tsetse area has been described by Bedford (1936a), Buxton (1948, 1949), Evans (1948, 1949, 1950a, b), Lewis (1949) and previous writers mentioned by him, and by van Saceghem (1916), and in the Sudan Government's (1948) report, pp. 189, 192, and the annual reports of the Sudan Veterinary Service.

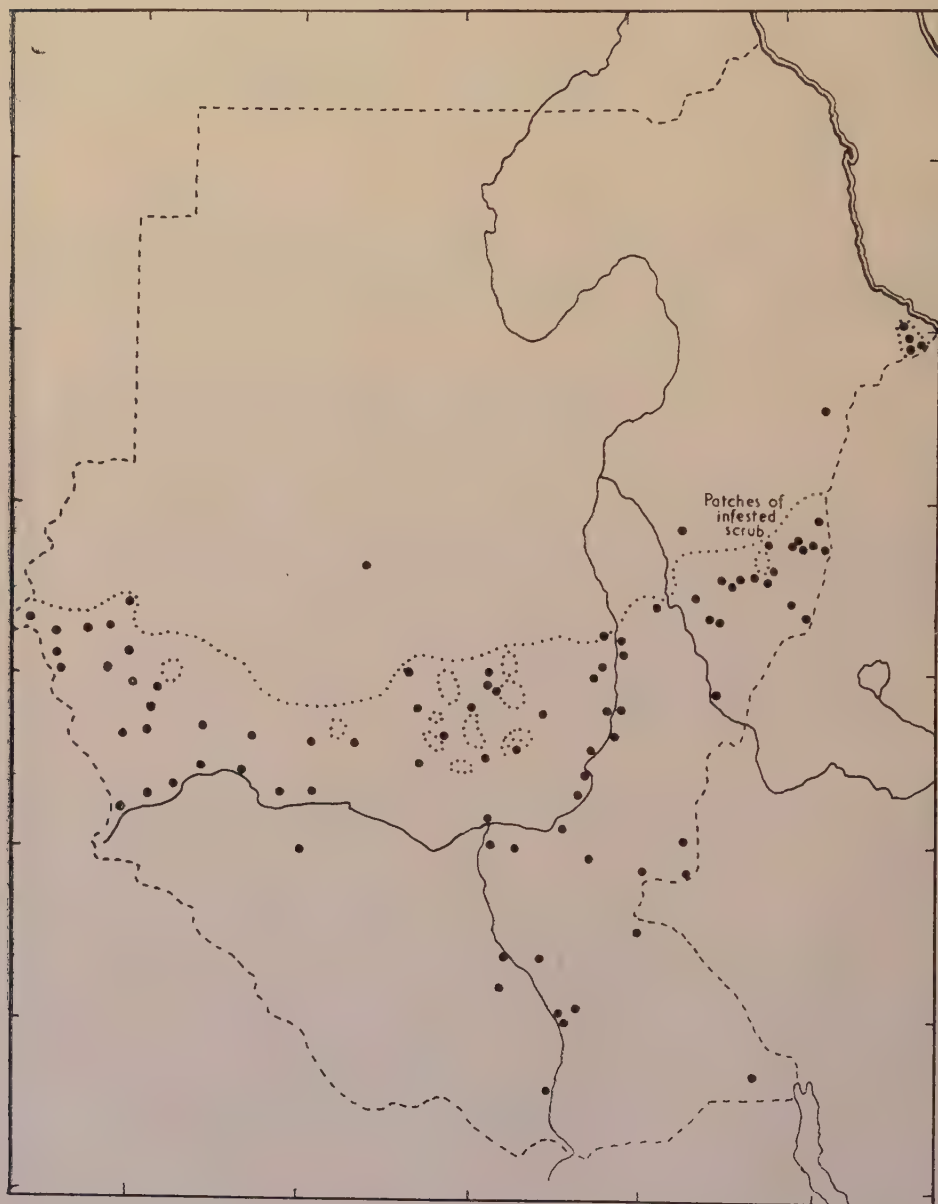


Fig. 26.—Showing places where Tabanids have been reported to be troublesome, and, approximately, the northern limit beyond which they are not generally troublesome to cattle in the rains.

Trypanosomiasis outside the tsetse area is due mainly to *Trypanosoma congolense* and partly to *T. vivax*. It is believed to be introduced from the tsetse area by migrating cattle and game, to be transmitted by Tabanids and possibly other biting flies, and to be further spread by the extensive annual movement of cattle. The disease has been known since 1906, and its incidence increased between 1934 and 1946 when closer administration and the immunisation of cattle against other diseases allowed more movement of trade cattle. The resulting increase of the disease probably caused mechanical transmission to become important, and there were several seasons of heavy rain in which Tabanids were unusually plentiful. By the dry season of 1946-1947 an enormous area on the White Nile and its tributaries between 6° and 13° north was affected. It covered more than 100,000 square miles and carried about one and a half million cattle. Most herds were infected and many lost more than half their animals. At one time the death rate was estimated to exceed 10,000 a month. Cases of cattle trypanosomiasis occasionally occur as far north as Gedaref, the Gezira, Kadugli, the Kassala area, Kosti and El Obeid.

It is not unlikely that Tabanids play a double part in spreading the disease, by transmitting it from one animal to another and by causing the animals to migrate and so spread the disease still further.

Trypanosomiasis of equines.

Many cases occurred in the tsetse areas before motor transport was widely used.

Trypanosomiasis of camels.

Much information has been given by Austen (1909), Balfour (1906, 1908a,b), Bennett (1929, 1933), Fry (1911), Hoare (1940, 1947), Hoare and Bennett (1937), King (1908), Knowles (1927) and Leese (1927), and in the Sudan Veterinary Service annual reports.

The disease, known as *gufar* in Arabic, is closely allied to the Indian surra and is caused by *Trypanosoma evansi*, but in the Sudan it does not normally affect animals other than the camel, of which it was the principal disease in the Sudan and is still important. The enzootic area extends across the Sudan approximately between latitudes 13° and 18° north, and its northern limit corresponds roughly with the 300 mm. isohyet. It is that part of the camel country (the southern part) which lies in the Tabanid area. A few cases are found further afield, in camels which have travelled from infected places. The limits of the camel trypanosomiasis area vary somewhat, perhaps one degree north or south in certain years. The disease is much dreaded by camel owners owing to the very heavy annual losses which it can cause.

The nomad camel owners consider that Tabanids transmit the disease. *Tabanus taeniola* is thought to be the vector in most areas. *T. sufis*, *Stomoxys* and other flies have been suspected in some districts, *Ancala africana* on the White Nile and *Pangonia magretti*, *T. leucostomus* and *T. mordax* near Karora. *P. magretti* has been thought to transmit the disease in the plains, but in one area it is reported to cause so much annoyance that camels are grazed at night and protected by smoke fires by day and therefore unlikely to be infected by this fly. In 1938 some camels were reported to have become infected in the dry season at a place where no biting flies were found.

Before drugs were available the main method of prevention in the Sudan, as in other camel countries (Leese, 1927), was to move the camels northward out of the Tabanid area during the rains. By 1933 the development of diagnosis and treatment had made it possible to prevent very heavy losses and to maintain police camels in the Tabanid districts during the rains, and in 1946 some 67,000 doses of "Antrypol" were issued. In 1945 the existence of a trypanosome strain resistant to this drug was confirmed and since then others have been tested.

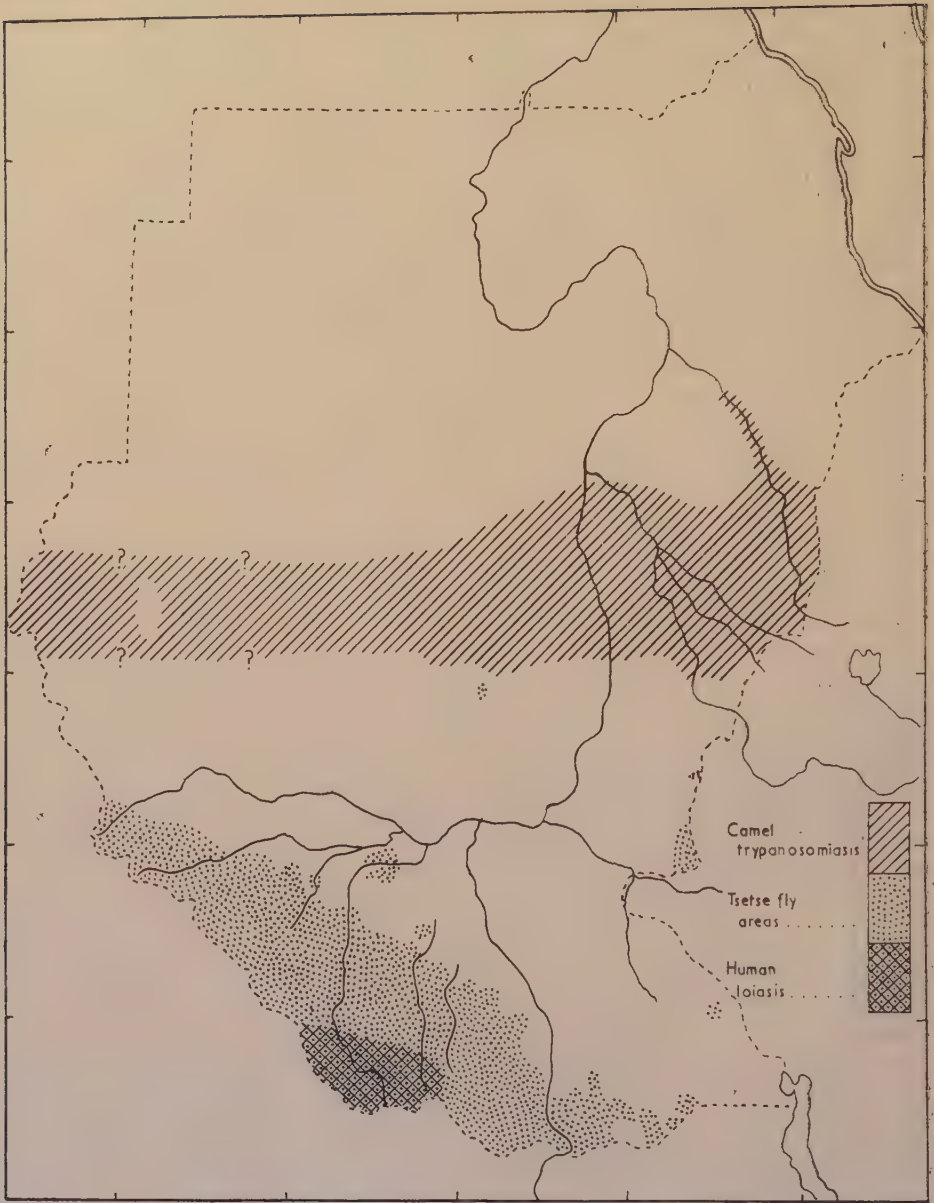


Fig. 27.—Showing approximately the distribution of camel trypanosomiasis (mainly after Hoare & Bennett, 1937, and Hoare, 1940), human loiasis (after Woodman, 1949), and tsetse flies (after Lewis, 1949).

The disease also occurs in the adjoining countries, Eritrea and Egypt, and other parts of North Africa (Austen, 1909 ; Di Domizio, 1918 ; Efflatoun, 1930 ; Ferraro, 1917 ; Martiglio, 1913 ; Postiglione, 1935 ; Pricolo & Ferraro, 1918). In Eritrea species of *Tabanus* in particular, and *Pangonia* and *Stomoxys* are thought to be vectors, and camel owners avoid the fly-infested districts most of which are on mountain slopes facing the sea. *T. taeniola* is thought to be the chief vector in Egypt, and *Atylotus agrestis* has been suspected there and in Timbuctu.

Loiasis.

Cruickshank (1936) reported that as far as was known *Loa loa* occurred chiefly in the Zande country. This is on the edge of the forests of the West African Sub-region to which *Loa loa* is confined (Gordon & others, 1950). Woodman (1936, 1948, 1949) and Woodman and Bokhari (1941) studied the disease, chiefly in the Li Rangu area, and found that it did not extend beyond 6°N. or 30°E. It was

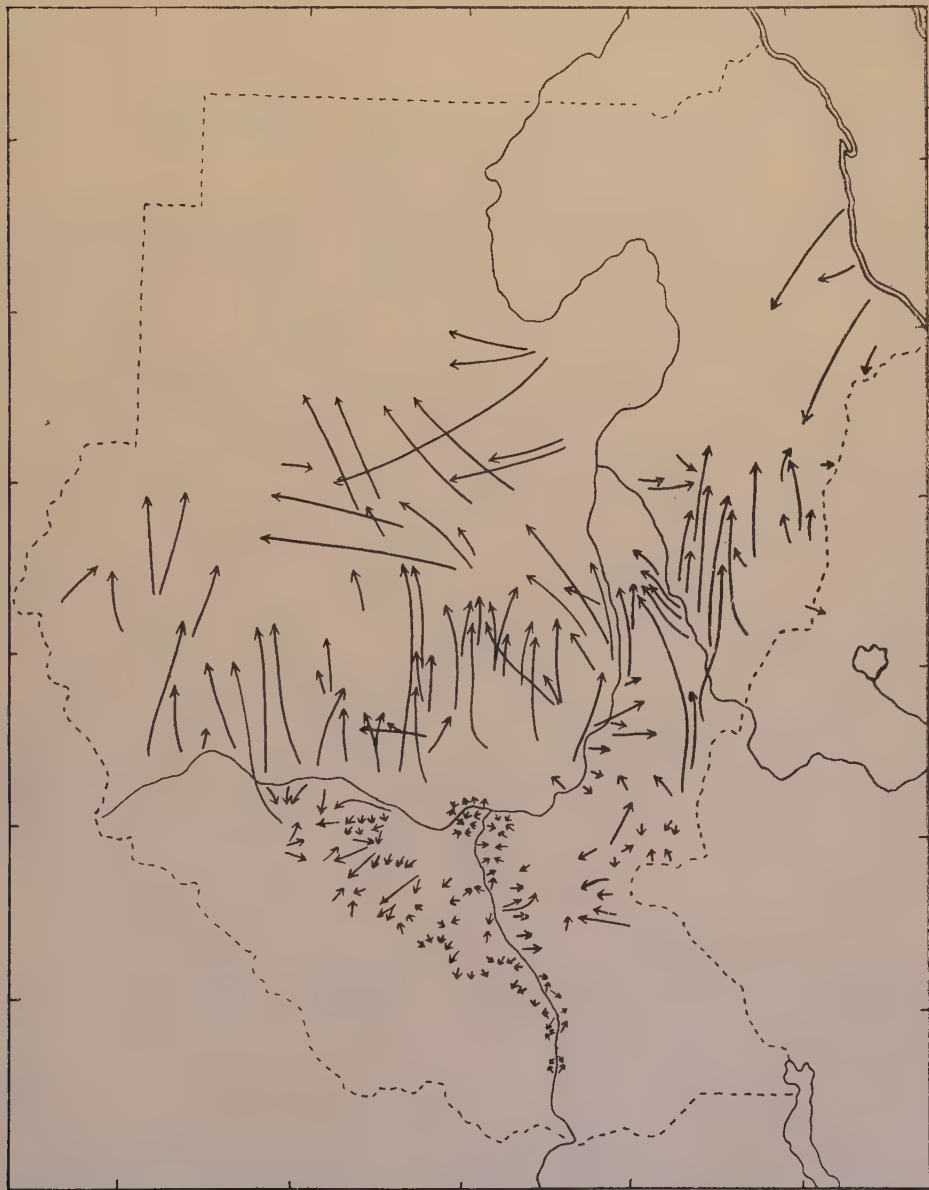


Fig. 28.—Showing approximately the main lines of the annual migrations of domestic animals during the rainy season, between May and September.

estimated that 20 per cent. or more of the Zande and, in 1934, 23 per cent. of the Europeans were infected. Infection experiments were carried out with the prevalent species of *Chrysops*, *C. distinctipennis* and *C. longicornis* (which was about 26 times less common), and 600 caught flies were dissected of which 0.7 per cent. were found to be infected. It appeared that *C. distinctipennis* was a local vector but an unimportant one to judge from its low infection rate and furtive habits and the fact that it was seldom seen to bite man, although it was common on cattle. *C. silacea* was only found in one place, Sources Yubu, during five years' work, and only once in appreciable numbers when it was taken on cattle in the rains. The distribution of loiasis did not appear to correspond to that of *Chrysops* and it was thought that another fly, perhaps not a Tabanid, was the vector. Attempts to infect some common man-biting flies including *Haematopota brunnescens*, *H. decora* and *H. patelllicorne* suggested that these were not vectors, and *Hippocentrum strigipenne* (a vicious biter in West Africa according to Bequaert, 1930, p. 97) and *H. versicolor*, which have a short seasonal incidence, were suspected.

Bloss (1949) commented that in his experience *Chrysops* did attack man, being a nuisance in the Li Rangu leprosarium and attacking carriers in the bush, and that the Zande knew these flies and had a name for them.

Woodman (1950) considered that the *Chrysops* population did fluctuate somewhat but that the evidence for another vector remained strong.

Dr. A. Bukhari sent the writer a collection of Tabanids for identification from Sources Yubu in 1950. They comprised nine *C. silacea* and one *Tabanocella perpulcra*. He reported that the flies had been abundant in 1936 but had gradually disappeared, and that a few were seen on cattle towards the end of 1949 and many in 1950 late in the afternoons.

The present writer has only spent a few weeks in the loiasis areas, but has seen them in both the dry and rainy seasons and looked for *Chrysops* in the forest fringes of streams and in open country. Only one was seen, but this was *C. silacea*, on a forested stream at Yambio. It may be that this species is widely distributed in the loiasis area. Gordon and others (1950) found that in West Africa this species inhabits the forest canopy and attacks man where he is exposed to view, as for example where the trees are low or where there are small clearings among high trees. In the loiasis area of the Sudan the thick forest grows in strips along streams and there may be only certain places where man comes into view of the canopy.

The distribution maps (figs. 6 and 7) show that *C. silacea* is the only species confined to the loiasis area except *C. funebris*, which has only been found once, and *C. longicornis*, which is seen in small numbers. *C. distinctipennis* occurs mainly outside the loiasis area and, if it is an important vector, it is surprising that the disease is not more widespread.

The Distribution of Pastoral Tribes and the Annual Migrations of Animals.

The need to escape from Tabanids often causes, or helps to cause, numerous animals to migrate. These movements are an important feature of the life of the country. They determine the place of residence and the way of life of many people, and doubtless increase the spread of diseases.

The distribution of pastoral tribes.

During the thirteenth, fourteenth and fifteenth centuries the Arabs came and occupied the northern and central Sudan, and in the course of time merged to some extent with those of the former inhabitants who had not been forced into the hills and fastnesses of the south (MacMichael, 1922, p. 30; 1934). It appears that the Arabs, with camels and sheep but probably no cattle, spread south until they were

stopped by biting flies and other adverse conditions. *T. evansi* may have been an important influence if it existed at that time. Hoare (1940, 1947) has suggested that this parasite may have arisen from the tsetse-borne *T. brucei* when camels entered tsetse country. The camel-owning tribes now live between 13° and 18°N. because the fly-borne *gufar* kills camels south of 13° and lack of rain and grazing keeps them south of 18° (Reid, 1935).

Some of the original camel-owning Arabs probably acquired cattle after a time and spread south until they reached the great swamps and flood plains with their numerous biting flies and mosquitos and stopped at the line which is still the southern limit of the Arabs.

The Negroid inhabitants of these "fastnesses" south of about 10°N. own vast numbers of cattle. Only short seasonal migrations are possible because to the north the land is occupied by Arabs and to the south tsetse flies prevail. Yet the people manage to keep their cattle, which are somewhat resistant to the local conditions and are protected from insects by constant attention by day and night.

There are thus three main areas north of the tsetse country, which are inhabited principally by Nilotic cattle owners, Arab cattle owners and Arab camel owners, respectively. From what we know of the Tabanids it appears that they may have played a considerable part in establishing this distribution and the way in which the people live.

Migrations of domestic animals with their owners.

Fig. 28 shows the general direction and extent of the migrations undertaken each rainy season by many of the pastoral people with their animals. The map is based mainly on information given by Berry (1928), Evans-Pritchard (1933), Hodgkin (1951), Lampen (1950), Lloyd (1910), MacMichael (1922), Paul (1950), Tothill and others (1948, pp. 806, 807, 835, 866 and 867) and other authorities mentioned below. Some of the migrations are very extensive, that from K. Yabus to Manaql for instance, being over 500 km. (300 miles) each way. The distances vary somewhat from year to year according to rainfall.

The northern arrows indicate mainly the movements of camels which are taken north to find suitable grazing and, to some extent, to escape from trypanosomiasis and biting flies.

Arab cattle, whose movements are shown by arrows in the central Sudan, do not go so far north because they are less troubled by biting flies and because the trypanosomes which affect them are less prevalent and less easily transmitted mechanically than *T. evansi*. The cattle travel north to dry country, or sometimes to local high ground, largely to avoid biting flies, and return after the rains when pasture and water supplies fail. They are sometimes obliged to return south before the Tabanids have disappeared from flooded areas.

The Negroid Nilotics undertake short migrations to local high ground when their riverain pastures are flooded and infested with Tabanids and other biting flies.

There are at least three million cattle in the Sudan and nearly all of them are nomadic (Boyns, 1947). The numerous old publications listed by Lewis (in press) and those mentioned below and in the sections on areas indicate that Tabanids are an important cause of many migrations. In many of these publications "fly" is used for Tabanids outside the tsetse areas. According to Peel (1904) the "serut" made it impossible for camels and cattle to live south of 13° at certain seasons, and Gleichen (1905) refers to the northward movement away from Tabanids in several parts of the country. Balfour (1906) regarded *Pangonia* as an important cause of the movements of camels. Lampen (1933, 1935) considered that the northern "sandhills on which to escape from the fly when the mud plains are in flood" were one of the special conditions suitable for keeping cattle west of the Nile between

10° and 13°N. Atkey (1934) wrote of the nomads of the north and central Sudan whose "seasonal migrations are regulated by the rains, the grazing and the fly." The annual reports of the Sudan Veterinary Service contain several references to trypanosomiasis in cattle which have remained too long in the south, tempted by the luscious grass which follows the early rains. The report for 1929, when the rains were very heavy and blood-sucking flies extended further north than usual, mentions a heavy mortality in police camels which the tribes avoided by keeping their camels north of the Tabanid areas. The report for 1939 points out that nomad "camel owners who move their herds slowly northwards at the onset of the rains escape heavy infection."

The movements of wild animals.

Several observers have considered that Tabanids help to cause some of the migrations of game animals. Apart from old references quoted by Lewis (in press) and later ones mentioned in the present paper, Balfour (1906) considered that Tabanids drove game north from Jebelein in the rains and Tohill and others (1948, p. 147) mentioned wild game, as well as cattle, being driven north by biting flies.

Tabanids are known to cause movements of wild animals in some other countries (Cameron, 1926 ; Darling, 1941, 1947 ; Edwards & others, 1939, p. 70). In Canada, caribou migrate in summer to places where there are fewer Tabanids. In Scotland Tabanids, more than anything else, drive the red deer to high grazing in July. The deer may receive 30 bites a minute from *Haematopota* but it is the larger Tabanids which, partly by their buzzing, stampede the animals and may prevent grazing and milking. These flies are most active in sunlight between 10 a.m. and 4 p.m. in hot still weather. Reindeer are among the animals affected in Europe.

Control.

The development of the country is likely to increase the need for controlling Tabanids by increasing the demand for meat and by necessitating settlement in certain areas which have hitherto been largely evacuated in the rainy season.

Philip (1931) discusses the effects of drainage, agriculture, repellents, protective nets and shelters for pasturing stock, but no radical results have been achieved and, in the words of Matheson (1950), "No successful methods of controlling horseflies have as yet been devised." Measures against Tabanids have scarcely been attempted in the Sudan apart from the local custom of clearing bush in a few districts, the extensive seasonal migrations to escape attack, the use of smoke, and grazing animals at particular hours.

It is to be hoped that these large biting flies, which have marked preferences for certain types of vegetation, may prove susceptible to some of the control methods used against tsetse flies, and that the marked attraction of Tabanids to artificial structures may lead to an effective method of trapping.

The following are some instances of attempts to achieve protection against Tabanids.

Smoke is often used, particularly by the southern Nilotics. Lampen (1933) referred to its use in "cattle camps" during the rains. Donkeys are kept in smoke-filled huts at Er Ruat and many other places from which cattle are taken north.

Some individuals have used mosquito nets and mosquito boots in heavily infested places.

Oil from the seeds of mahogany (*Khaya senegalensis* A. Juss.) is said to be used for keeping Tabanids off cattle in the south (Anon., 1911 ; Broun & Massey, 1929). The writer's experience of repellents against Tabanids is confined to two females of

T. taeniola which were placed in a tumbler and held against an arm liberally smeared with liquid dimethyl phthalate. They immediately bit through the repellent.

At Geigar Tabanids are still present when cattle return southwards in September, and the animals are therefore only watered in the morning and evening. Sometimes the Dinka cattle at Renk are grazed only at these times.

An attempt to keep cattle free from trypanosomiasis near Kadugli was reported by Bedford (1937) and in the Sudan Veterinary Service report for 1935. The animals were kept at a distance from local cattle and the results were encouraging but inconclusive.

Notes on Areas.

The Watershed Area.

Along the Nile-Congo watershed (fig. 25) many of the streams are fringed with dense tropical rain forest. The area contains the Zande agricultural development scheme, near Yambio and the western part of the Zande tribal area. Reference to the map shows that several species of Tabanids are restricted to this area. According to Woodman and Bokhari (1941), *C. distinctipennis* is evenly distributed in the western two-thirds of the Zande tribal area and occurs at all seasons. The existence of some small permanent swamps probably accounts for the presence of *T. thoracinus*. Loiasis is exceedingly common in the Zande area (Abbott, 1950).

The Raga-Loka area.

This is ironstone country with broad-leafed woodland. There are many seasonal streams but no swamps. The Tabanids, which are not usually troublesome, are largely species of *Haematopota*.

The Imatong Area.

Thick rain forest grows on parts of the mountains. Several mountain species have been found, of which *H. ugandae* is common.

The Torit-Loelli Area.

This is rather a dry region of which the vegetation is Acacia Short Grass Scrub (Tothill & others, 1948) similar to those of the adjacent part of northern Kenya and of the north-eastern part of the Central Rainlands area much further north in the Sudan. The presence of *T. taeniola* and particularly of *P. magretti* and *H. taciturna* emphasise the similarity of the Torit-Loelli area to northern regions.

Bimbashi C. J. Lambert has noticed that between Loelli and Moru Ethi in 1950 *T. taeniola* was present before the herds of cob (*Kobus* sp.) arrived at the beginning of May and was then replaced by large numbers of *P. magretti*.

The Boma-Gemi Area.

This series of small hilly areas consists of outliers of the mountains of Ethiopia. *H. ugandae* is among the few Tabanids that have been collected. Records from across the frontier are *C. stigmatalis* Loew from Maji (2,320 metres) and *H. remota* Oldroyd from Mashi, both taken by Dr. E. K. Malone in August, 1941.

The Flood Plain Area.

This vast area of clay plain is subject to extensive shallow flooding by rain and river floods but most of it is waterless in the dry season. The type of country which is annually flooded by a river is known locally as *Toich*. The vegetation of the Flood Plain Area is grass with patches of bush on higher ground. It is inhabited by the cattle-owning Nilotic tribes, Dinka, Nuer and Shilluk.

The principal species are *C. distinctipennis*, *Ancala africana*, *A. fasciata* var. *nilotica*, *A. latipes*, *Atylotus agrestis*, *A. fuscipes*, *T. biguttatus*, *T. par*, *T. taeniola* and *H. brunnescens*. Tabanids are numerous at many places. In the Nuer country they are troublesome from May to July or September, and sometimes in other months (Evans-Pritchard, 1940). Titherington (1927) mentions "large stoat flies" which accompany hippopotamus and elephant and are apt to be a nuisance locally in the western Dinka country.

Evans-Pritchard (1938) has described the annoyance caused by Tabanids in the Nuer area, which they are known as *rom* and are troublesome on cloudy days. At Yakwac and Konye "they appeared in swarms every morning shortly before the cattle were driven to pasture, between 9 and 9.30 a.m.," when Dr. Evans-Pritchard was driven from his tent by their attacks. The flies accompanied the cattle to pasture. "On most mornings at Konye about two hours after being driven to pasture the herd came stampeding back kicking their legs and lashing out with their tails, and dung fires had to be lit in the kraal to protect them. There was no need to tether the cattle for they were only too grateful to be allowed to stand near the fires and let the smoke envelop them. Only when there was a shower of rain did they get any relief in the pastures for rain drives seroot away. On cloudy rainless days the poor beasts ran back to their village covered with seroot and their flanks stained with blood. On such days they were not able to graze for more than two or three hours."

Most of the cattle trypanosomiasis of the Sudan occurs in the Flood Plain Area.

From June to September the Nuer country is inundated and the people retire to patches of dry ground where they are less affected by biting flies. The cattle appear to withstand the attacks of mosquitos, *Stomoxys* and Tabanids partly by their remarkable hardiness and endurance and partly because the Nuer live in a symbiotic relationship with their cattle in one single community and give them constant attention (Evans-Pritchard, 1940). Most of the Nilotic people in the Flood Plain Area are somewhat nomadic and take their livestock in the dry season to cattle camps on low-lying pastures which are inundated when the rivers are in flood. At the onset of the rains they return to their villages on higher ground which then become islands (Anon., 1926; Tothill & others, 1948, pp. 652, 693).

North of the Nilotic tribes, the Baggara Arabs camp along the north bank of the Bahr el Arab in the winter, and, when the rains come move north because the ground becomes swampy and biting flies attack the cattle (Hodgkin, 1951).

Conditions will one day be profoundly altered in parts of the Flood Plain Area, in the Sudd Area, and on the White Nile down to Kosti by the construction of the Jonglei canal between Jonglei and the Sobat mouth and by dams to be built in Uganda (Sandon, 1951; Sudan Government, Jonglei Team reports, 1946 to 1948; Worthington, 1950, p. 38). The main effect will be to reverse the flood seasons in the southern part of the Sudd and to maintain the river at a permanent high level in the north. The natural grazing of nearly a million cattle will be affected, much of it being obliterated by flooding and much of the rest being rendered useless by biting flies breeding in the newly created swamps. An alternative system of husbandry is being planned to meet the new conditions.

The Sudd Area.

This consists largely of a vast permanent swamp overgrown with papyrus and *Vossia* grass. The name is derived from the Arabic word *sadd*, a barrier, owing to the frequent blocks which were caused by floating vegetation in past years.

Among the permanent swamps between Shambe and Lake No, *C. brucei*, *T. par* and *T. thoracinus* are the principal species. In the country which is only seasonally flooded the Tabanid species of the flood plains are found. *T. taeniola* can be abundant at Buffalo Cape where the *toich* is in contact with the river. King (1910a) reported

that *T. par* was numerous near Bor, and Comyn (1911) encountered many *surret* at Lake No.

The Nuba Area.

In this area there are numerous rocky hills, some with permanent streams, surrounded by flat country. In the hills several southern species of Tabanids are found, and in the plains northern species occur, particularly *P. magretti* which is troublesome and is locally known as *umm bum*.

During the rains many of the local breed of cattle owned by the sedentary Negroid Nuba tribes are protected from biting flies by smoke fires and others are kept on high ground, while the cattle of the semi-nomadic Arab tribes are taken to the sandy country (Colvin, 1939).

The Central Rainlands Area.

This is a large area, mainly of clay, north of the flood plains. Its northern boundary approximates in places to the junction between clay and sand and in places to the 500 mm. isohyet. The vegetation is largely Acacia Tall Grass Forest with some Acacia Short Grass Scrub in the north-east. In this rather flat country with an impermeable soil and no artificial drainage many swamps appear in the rains and Tabanids abound. *P. magretti*, *Atylotus agrestis*, *A. fuscipes*, *T. gratus*, *T. sufis* and *T. taeniola* are the principal species. Several southern species extend down the White Nile into the area.

Tabanids are widely distributed inland and common on the White Nile. They are noticeable south of Kosti (Boulenger, 1907) and abundant at Jebel Ahmed Agha (Chapman, 1921) and many other places. A steamer in which the writer was travelling was boarded in the midstream by *T. taeniola* near Juri, and those of the crew not on duty sheltered behind mosquito wire.

Cattle trypanosomiasis varies in extent. For example, near the R. Rahad it appeared in 1944 for the first time since 1928 (Sudan Vet. Serv., Report for 1928). Camel trypanosomiasis is widespread. Reid (1930) wrote of the White Nile area where a camel which had recovered was "exceedingly valuable in the south where the rains are heavier and consequently the fly more prevalent." According to Lloyd (1910) camels are in danger of being infected by *serut* (*P. magretti*) south of El Obeid.

There are many references to the effect of Tabanids on the movements of animals. Reid (1930) described the migrations of the Baggara (semi-nomadic cattle-breeding Arabs) on the White Nile. "During the rains the Baggara move away from the river and the wells. The extent of their migration is governed by the "fly" and the rains. If the fly is really bad, a tribe like the Gima'a will reach Jebel Bachi, and have been known to reach Jebel Arashkol, but ordinarily speaking they seldom move more than a day or two from their summer quarters. The Seleim and Dar Muharib, who graze mostly near the river, have a wider range owing to the greater prevalence of the fly, etc." Tothill and others (1948, pp. 805, 806), referred to the semi-nomadic people of the Kosti district migrating to avoid "fly" and returning to obtain water, flocks ranging as far north as northern Kordofan and as far south as Renk.

The cattle of the Kenana district graze mainly in the Jebel Moya area in the rains when the southern reaches of the Blue Nile are practically inaccessible owing to floods, flies and mosquitos (Scott, 1947). The Arabs of the Fung (the upper Blue Nile Area) west of the Ingessana Hills start north for the southern Butana when the flies increase (Hodgkin, 1951). Camels from the Rahad and Dinder "move north into the Butana in the rains to avoid the fly" (Tothill & others, 1948, p. 805). Some wait for good grazing to appear south of the R. Dinder but must cross it before it rises in flood or else their camels would be cut off and attacked by *surret*.

Further east, according to Tothill and others (1948, p. 730), one would expect to find mixed husbandry well developed, but the "various biting flies which follow the game migration northward at the break of the rains make much of the agricultural part of the district unsuitable for cattle in the rains, and the sedentary cattle-owning cultivators are forced to drive part at least of their herds north with the nomads, although use is made of certain hills and open plains (known to be habitats not favoured by the flies) to keep milking animals within reach during the rains."

With regard to the country along the R. Atbara, Hayes (1905) wrote of the great annual incursion of Arabs into the Goz Regeb area, to which the provincial Government then used to be transferred, and emphasised the desirability of controlling "serrut."

A method of cultivation known as *harig* (from *hariga*, a fire) is practised over large areas in association with the annual migration. It is "a controlled burning of grass in the early rains, to kill both the young and old grass and weeds, followed by planting to dura", and "is well suited to the nomad tribes who trek northwards in July and August when the biting flies make life difficult for their animals" (Keen, 1946). Worthington (1946) described *harig* cultivation as "an excellent example of an ecological relation between nomad cultivators, an insect, and special plant communities". "The nomads move northwards through the area in the summer, being driven out of their winter grazing-lands by vast numbers of horse flies which come with the rains . . . travelling back through the same country in autumn the crop is harvested".

The Tabanids of the Central Rainlands Area are likely to cause additional trouble when economic development takes place. There is sufficient rain for crops to grow but not enough for a population of cultivators. Water is now being provided by means of large mechanically excavated *hafirs* or storage tanks in the ground. Cultivators will increase in relation to the nomadic pastoralists (Jefferson, 1949, p. 86), and it remains to be seen if mixed farming can develop in country where migration is at present the only effective large scale method of protecting cattle from Tabanids. In the words of Tothill and others (1948, p. 731) "Studies in the identity and distribution of biting flies, and in the protection value of clearances such as the grass plains (or "sagea"), are needed before an improved cattle husbandry can be initiated. Save for an occasional water-wheel on the Rahad, cattle are not used in transport or in cultivation, and development of their use is one of the greatest contributions which the future holds for the expansion of production in the rainlands". In the reports of the Sudan Government Soil Conservation Committee (1944, p. 88) it is recommended that as many cattle should be kept as village water supplies permit and that they should be allowed access to less fly-infested districts in the rains.

The Gezira Area.

The Gezira, meaning island in Arabic, is the country bounded on the east, west and north by the Blue and White Niles and on the south by an indefinite line between Kosti and Sennar. It has been much altered by irrigation. In fig. 25 the northern limit of the Acacia Short Grass Scrub (shown by Tothill & others, 1948) is disregarded in the Gezira Area which is drawn to include Khartoum and two large reservoir areas.

Tabanids are not common in most parts of the Gezira. At Wad Medani a few *T. gratus*, *T. sufis* and *T. taeniola* are to be seen in the wet and dry seasons. They probably breed in irrigation water.

The Fasher-Butana Area.

The vegetation of this area is largely Acacia Desert Scrub. It includes the Butana, a rather dry sandy region between Kassala, Wad Medani and Khartoum.

Tabanids are unknown over most of the area but are abundant in patches of scrub in the Butana during the rains. Loaded camels are only taken through these patches by night because they roll on the ground when attacked, and even people travelling by car have had to make wide detours or wait till nightfall. An isolated locality for *P. magretti* is Hafir Abu Zemain where it was reported in September, 1949, a year of exceptionally heavy rain. The flies were said to remain near long grass and a camel party had to pass the area by night. According to Acland (1932) the vectors of camel trypanosomiasis appear in the eastern Sudan only where trees or high grass abound. Another instance of Tabanids being common unusually far north is mentioned in the Sudan Veterinary Service Report for 1936 "It has been customary for many years to withdraw Eastern Arab Corps and police camels from posts where biting flies are known to be present during the rains, usually from June or July to the end of October, and to keep them in the neighbourhood of Kassala. This year, however, unusually heavy infection occurred whilst the camels were grazing in an area near Kassala hitherto considered comparatively free from fly. It is the opinion . . . that Tabanid flies greatly increased in the area during 1936 owing to abnormal rainfall and a gradual increase in afforestation."

The southern part of the Fasher-Butana Area is a refuge to which animals are brought from the south during the rains. The cattle-owning nomads of southern Darfur range as far north as 13° and then south to the Bahr el Arab "till the rains and the fly again drive them north" (Tothill & others, 1948, p. 855). The Baggara Arabs from the Bahr el Arab are safe from biting flies north of 12°. According to Tothill and others (1948, p. 805) "The Rufaa district has a considerable livestock population and the camels of the Shukriya range far into the Butana during the rains. The camels from the Rahad and Dinder also move north into the Butana in the rains to avoid the fly."

The Marra Area.

This area includes the Marra Mountains among which some southern species of Tabanids have been recorded but are not known to be numerous. *Haematopota* has been reported to be a serious pest near Zalingei where it is locally known as *dimbeiri* (Arabic) or *gobdi* (Fur) and occurs chiefly near *Anogeissus* trees. For years the fly was seen near Gallabat (Darfur Province) and in 1939 spread to the Kas-Zalingei road. In August, 1940, it made the road almost useless for animals by day, and some donkeys which travelled along it died afterwards.

The Baiyuda Area.

Tabanids are unknown in the desert but a few have been recorded on the Nile.

The Beja Area.

This area includes the Red Sea Hills and the coastal plain where rain falls in the winter. In the past the Eritrean border region near Kassala has been included in the northern Sudan by some naturalists and in the southern Sudan by others. In the present paper (fig. 25) it is placed in the Beja Area (part of the North-East African Province) which is extended north to the Egyptian border.

Several species of Tabanids occur in this area but not elsewhere in the Sudan.

Chapman (1921) has written of Tabanids in the Red Sea Hills. He found that camels could not withstand their attacks and he himself was unable to visit ibex country in the Karora hills in April because they were then infested by a Tabanid which drove man and beast to lower levels. He described the annual migrations of ariel, *Gazella soemmerringii soemmerringii* (Cretzchmar) or var., and wrote: "Another predisposing cause for seasonal movements—perhaps more important even than food supply—is a seroot fly of sorts, which in spring invades the higher ground in ferocious swarms which drive both game and Arab herdsmen, along with their flocks, pell-mell from the hills."

The Palaearctic Area.

This is nearly all desert and Tabanids are unknown.

Summary.

Seventy species of Tabanids occur in the Sudan. Many of the inhabitants are pastoralists, and the Tabanids have a profound effect on them, largely by causing them to remove their animals from infested areas in the rains and to undertake extensive annual migrations. New problems will arise when economic development involves permanent settlement of certain infested areas. This paper sets out the problem, and satisfactory control measures have yet to be devised.

The country is briefly described.

The section on individual species consists mainly of lists of localities which are shown on maps.

In a general discussion of distribution faunal areas are designated for discussing Tabanids in the Sudan. The composition of the Tabanid fauna, which is mainly Ethiopian, is described. Types of habitat are indicated, and distribution is discussed in relation to systematic position. The area in which Tabanids are abundant, and their seasonal prevalence, are indicated.

There are brief references to habits, particularly the attraction to inanimate objects and the fact that some species bite man on some occasions and not others.

The economic effect, namely, the direct effect of bites and probable role of the flies in the transmission of cattle and camel trypanosomiasis and human loiasis, are described, mainly from published sources.

The effect of Tabanids on the distribution and annual migrations of pastoral tribes is described. Attacks by flies on domestic animals and the search for pasture and water are the main causes of the very extensive rainy season migrations which are shown on a map.

The problem of control is very briefly discussed.

Additional information on some of the foregoing subjects is given in notes on faunal areas.

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References.

This list includes several papers in which Sudan species were described.
ANON. (1911). The Bahr el Ghazal Province. A.-E.-Sudan Handb. Ser. 1, London, H.M.S.O.

- ANON. (1926). Report on the . . . administration . . . of the Sudan in 1925.—London, H.M.S.O.
- ABBOTT, P. H. (1950). A survey of signs of nutritional ill-health among the Azande of the southern Sudan.—Trans. R. Soc. trop. Med. Hyg., **43**, pp. 477–492.
- ACLAND, P. B. E. (1932). Notes on the camel in the eastern Sudan.—Sudan Notes, **15**, pp. 119–149.
- ARCHIBALD, R. G. (1912). A trypanosome of cattle in the southern Sudan.—J. comp. Path., **25**, pp. 292–297.
- ATKEY, O. F. H. (1934). The distribution of leprosy in the Sudan with reference to climate and diet.—Int. J. Leprosy, **2**, pp. 193–200.
- AUSTEN, E. E. (1903). A monograph of the tsetse-flies . . .—319 pp. London, Brit. Mus. (Nat. Hist.).
- AUSTEN, E. E. (1906). On some blood-sucking and other Diptera from the Anglo-Egyptian Sudan . . .—2nd Rep. Wellcome trop. Res. Lab., pp. 51–66.
- AUSTEN, E. E. (1908a). New African phlebotomic Diptera in the British Museum (Natural History). Part I. Tabanidae.—Ann. Mag. nat. Hist., (8) **1**, pp. 209–228.
- AUSTEN, E. E. (1908b) . . . Part II. Tabanidae (continued).—*Ibid.*, pp. 401–428.
- AUSTEN, E. E. (1908c) . . . Part IV. Tabanidae (continued).—*Ibid.*, **2**, pp. 274–301.
- AUSTEN, E. E. (1909). Illustrations of African blood-sucking flies other than mosquitoes and tsetse-flies.—221 pp. London, Brit. Mus. (Nat. Hist.).
- AUSTEN, E. E. (1910). New African phlebotomic Diptera . . . Part VII. Tabanidae (continued).—Ann. Mag. nat. Hist., (8) **6**, p. 337–356.
- AUSTEN, E. E. (1911a). A new species of *Tabanus* from the Anglo-Egyptian Sudan.—Bull. ent. Res., **1**, pp. 291–293.
- AUSTEN, E. E. (1911b). Two new species of *Tabanus* from the Anglo-Egyptian Sudan.—*Ibid.*, **2**, pp. 173–177.
- AUSTEN, E. E. (1912a). New genera and species of Tabanidae in the British Museum (Natural History).—Ann. Mag. nat. Hist., (8) **9**, pp. 1–33.
- AUSTEN, E. E. (1912b). New African species of *Tabanus*. Part I.—Bull. ent. Res., **2**, pp. 279–290.
- AUSTEN, E. E. (1920). A contribution to knowledge of the Tabanidae of Palestine.—Bull. ent. Res., **10**, pp. 277–321.
- AUSTEN, E. E. (1926). Zoological results of the Swedish Expedition to Central Africa 1921. Insecta. 19. Tabanidae et Glossininae (Diptera).—Ark. Zool., **18** B, no. 6, 4 pp.
- AUSTEN, E. E. (1939). Gad-flies. In Edwards, F. W., Oldroyd, H. & Smart, J. British blood-sucking flies, pp. 149–152. London, Brit. Mus. (Nat. Hist.).
- BALFOUR, A. (1904). Biting and noxious insects other than mosquitoes.—1st Rep. Wellcome trop. Res. Lab., pp. 38–39.
- BALFOUR, A. (1906). Biting and noxious insects other than mosquitoes.—2nd Rep. Wellcome trop. Res. Lab., pp. 29–50.
- BALFOUR, A. (1908a). Introduction.—3rd Rep. Wellcome trop. Res. Lab., pp. 15–25.
- BALFOUR, A. (1908b). Trypanosomiasis in the Anglo-Egyptian Sudan.—*Ibid.*, pp. 27–35.

- BALFOUR, A. (1911). Veterinary notes.—4th Rep. Wellcome trop. Res. Lab., A, pp. 343–352.
- BECKER, T. (1923). Wissenschaftliche Ergebnisse der von Werner unternommenen zoologischen Expedition nach dem Anglo-Aegyptischen Sudan (Kordofan) 1914. VI. Diptera.—Denkschr. Akad. Wiss. Wien, **98**, pp. 57–82.
- BEDFORD, H. W. (1936*a*). Entomological Section Agricultural Research Service. Veterinary Entomology.—Rep. agric. Res. Serv. Sudan, 1935, pp. 94–95. (Rev. appl. Ent., (B) **25**, p. 78.)
- BEDFORD, H. W. (1936*b*). Report on Medical Entomology.—Rep. Sudan med. Serv., 1935, pp. 80–82.
- BEDFORD, H. W. (1937). Entomological Section. Agricultural Research Service. Veterinary Entomology.—Rep. agric. Res. Serv. Sudan, 1936, pp. 51–52. (R.A.E., (B) **26**, p. 174.)
- BEDFORD, H. W. (1938). Medical entomology.—Rep. Sudan med. Serv., 1937, pp. 77–83.
- BENNETT, S. C. J. (1929). Camel trypanosomiasis in the Sudan.—Pan.-Afr. agric. vet. Conf., Pretoria, 1929, Pap. vet. Sect., pp. 10–15.
- BENNETT, S. C. J. (1933). The control of camel trypanosomiasis.—J. comp. Path., **46**, pp. 67–77, 174–185.
- BEQUAERT, J. (1930). Medical and economic entomology.—In Strong, R. P. *Ed.* The African Republic of Liberia and the Belgian Congo., **2**, pp. 797–1001. Cambridge, Mass., Harvard Univ. Pr.
- BERRY, W. J. (1928). The Arabs of Kordofan: a study in adaptation.—Scot. Geogr. Mag., **44**, pp. 278–292.
- BLOSS, J. F. E. (1949). Filaria in the Sudan.—Trans. R. Soc. trop. Med. Hyg., **43**, pp. 236–238.
- BOULENGER, G. A. (1907). The fishes of the Nile.—In Anderson, J. *Zoology of Egypt*, **3–4**, 578 pp. London.
- BOYNS, B. M. (1947). Sudanese cattle as milk producers.—Emp. J. exp. Agric., **15**, pp. 27–41.
- BROUN, A. F. & MASSEY, R. E. (1929). Flora of the Sudan. London.
- BUXTON, P. A. (1948). Trypanosomiasis in eastern Africa, 1947.—44 pp. London, Colon. Off.
- BUXTON, P. A. (1949). Notes on trypanosomiasis and tsetse in the southern parts of the Anglo-Egyptian Sudan.—[Publ.] Bur. interafr. Tsétsé, Léopoldville, no. 85, 11 pp., multigraph.
- CAMERON, A. E. (1926). Bionomics of the Tabanidae (Diptera) of the Canadian prairie.—Bull. ent. Res., **17**, pp. 1–42.
- CARPENTER, G. D. H. (1925). A naturalist in East Africa. . . —187 pp. Oxford, Clarendon Pr.
- CHAPMAN, A. (1921). Savage Sudan, its wild tribes, big-game and bird-life. London.
- COLVIN, R. C. (1939). Agricultural Survey of Nuba Mountains. Khartoum.
- COMYN, D. C. E. (1911). Service and sport in the Sudan. London.
- CRAWFORD, J. C. (1911). Descriptions of new Hymenoptera. 2.—Proc. U.S. nat. Mus., **40**, pp. 439–449.
- CRUICKSHANK, A. (1936). Tropical diseases of the southern Sudan: their distribution and significance.—E. Afr. med. J., **13**, pp. 172–177.

- DARLING, F. F. (1941). A herd of red deer ; a study in animal behaviour.—2nd edn. Oxford.
- DARLING, F. F. (1947). Natural history in the highlands and islands.—303 pp. London.
- DI DOMIZIO, G. (1918). Una tripanosomiasi del dromedario eritreo (Gudhò). Cenni sulle mosche ematofage della Colonia Eritrea.—*Clin. vet.*, Milano, **41**, pp. 391–413. (R.A.E., (B) **7**, p. 125.)
- DREW, C. M. (1911). Final report of the Sudan Sleeping Sickness Commission, 1909–10.—*Bull. Sleep. Sickn. Bur.*, **3**, pp. 85–87.
- EAST AFRICA HIGH COMMISSION. (1951). Virus Research Institute Annual Report for 1950. Nairobi.
- EDWARDS, F. W., OLDROYD, H. & SMART, J. (1939). British blood-sucking flies.—156 pp. London, Brit. Mus. (Nat. Hist.).
- EFFLATOUN, H. C. (1930). A monograph of Egyptian Diptera. Part III. Family Tabanidae.—*Mém. Soc. ent. Égypte*, **4**, fasc. 1, pp. 1–114.
- ENDERLEIN, G. (1925). Studien an blutsaugenden Insekten. I. Grundlagen eines neuen Systems der Tabaniden.—*Mitt. zool. Mus. Berl.*, **11**, pp. 253–409.
- EVANS, J. T. R. (1948). *Trypanosoma congolense* infection in cattle in the Sudan : treatment with dimidium bromide (Phenanthridinium 1553).—*Vet. Rec.*, **60**, pp. 418–420.
- EVANS, J. T. R. (1949). Rinderpest and trypanosomiasis.—*Sudan wild Life*, **1**, pp. 7–9.
- EVANS, J. T. R. (1950a). Bovine trypanosomiasis in the Sudan : mass treatment with antrycide.—*Vet. Rec.*, **62**, pp. 59–60.
- EVANS, J. T. R. (1950b). Control of bovine trypanosomiasis in the Anglo-Egyptian Sudan.—[Publ.] Bur. interafr. Tsétsé, Léopoldville, no. 113/0, multigraph.
- EVANS-PRITCHARD, E. E. (1933). The Nuer : tribe and clan.—*Sudan Notes*, **16**, pp. 1–53.
- EVANS-PRITCHARD, E. E. (1938). Economic life of the Nuer : cattle. (Part II).—*Ibid.*, **21**, pp. 31–77.
- EVANS-PRITCHARD, E. E. (1940). The Nuer : a description of the modes of livelihood and political institutions of a Nilotic people. Oxford.
- FERRARO, G. (1917). I ditteri ematofaghi della Colonia Eritrea incriminati della trasmissione delle tripanosomiasi locali.—*Clin. vet.*, Milano, **40**, pp. 487–493.
- FRY, W. B. (1911). Animal trypanosomiasis in the Anglo-Egyptian Sudan.—4th Rep. Wellcome trop. Res. Lab., A, pp. 41–56.
- GLEICHEN, A. E. W., Count. *Ed.* (1905). The Anglo-Egyptian Sudan : a compendium prepared by officers of the Sudan Government. . . —2 vols. London.
- GORDON, R. M., CHWATT, L. J. & JONES, C. M. (1948). The results of a preliminary entomological survey of loiasis at Kumba, British Cameroons. . . —*Ann. trop. Med. Parasit.*, **42**, pp. 364–376.
- GORDON, R. M., KERSHAW, W. E., GREWE, W. & OLDROYD, H. (1950). The problem of loiasis in West Africa. . . —*Trans. R. Soc. trop. Med. Hyg.*, **44**, pp. 11–41.
- HAYES, A. J. (1905). The source of the Blue Nile. . . London.
- HOARE, C. A. (1940). Studies on the behaviour of *Trypanosoma evansi* in tsetse-flies with special reference to its phylogeny.—*Parasitology*, **32**, pp. 105–121.
- HOARE, C. A. (1947). Tsetse-borne trypanosomiasis outside their natural boundaries.—*In* Rodhain, J. *Liber Jubilare*, pp. 267–277. Brussels, Soc. belge Méd. trop.

- HOARE, C. A. & BENNETT, S. C. J. (1937). Morphological and taxonomic studies on mammalian trypanosomes. III. . . —Parasitology, **29**, pp. 43–56.
- HODGKIN, R. A. (1951). Sudan geography. London, Longman, Green.
- JEFFERSON, J. H. K. (1949). The Sudan's grain supply.—Sudan Notes, **30**, pp. 77–98.
- KEEN, B. A. (1946). The agricultural development of the Middle East.—126 pp. London, H.M.S.O.
- KING, H. H. (1908). Report on economic entomology.—3rd Rep. Wellcome trop. Res. Lab., pp. 201–248.
- KING, H. H. (1910a). Some observations on the bionomics of *Tabanus par*, Walker, and *Tabanus taeniola*, Pal. de Beauv.—Bull. ent. Res., **1**, pp. 99–104.
- KING, H. H. (1911a). Some observations on the bionomics of *Tabanus ditaeniatus*, Macquart, and *Tabanus kingi*, Austen.—*Ibid.*, pp. 265–274.
- KING, H. H. (1911b). Report of the Entomological Section. . . —4th Rep. Wellcome trop. Res. Lab., B, pp. 95–150.
- KING, H. H. (1914). Further notes on the bionomics of *Tabanus ditaeniatus*, Macq., and *Tabanus taeniola*, P. de B.—Bull. ent. Res., **5**, pp. 247–248.
- KING, H. H. (1926). A note on the bionomics of *Tabanus fasciatus niloticus*, Aust.—*Ibid.*, **16**, p. 359.
- KNOWLES, R. H. (1927). Trypanosomiasis of camels in the Anglo-Egyptian Sudan : diagnosis, chemotherapy, immunity.—J. comp. Path., **40**, pp. 59–71, 118–143.
- KRÖBER, O. (1925). Egyptian Tabanidae.—Bull. Soc. ent. Égypte, **9**, pp. 77–137.
- KRÖBER, O. (1927). Die Chrysopsarten Afrikas.—Zool. Jahrb., Abt. I. Syst., **53**, pp. 175–268.
- KRÖBER, O. (1929). Neue Dipteren aus Aegypten aus den Familien Tabanidae, Therevidae, Omphralidae u. Conopidae.—Bull. Soc. ent. Égypte, **13**, pp. 73–84.
- KRÖBER, O. (1939). Katalog der palaearktischen Tabaniden. . . —Acta Inst. Mus. zool. Univ. Athenien, **2**, pp. 57–245.
- LAMPEN, G. D. (1933). The Baggara tribes of Darfur.—Sudan Notes, **16**, pp. 97–118.
- LAMPEN, G. D. (1935). The Baggara tribes.—In Hamilton, J. A. de C. Ed. The Anglo-Egyptian Sudan from within, pp. 130–139. London.
- LAMPEN, G. D. (1950). History of Darfur.—Sudan Notes, **31**, pp. 177–209.
- LEESE, A. S. (1927). A treatise on the one-humped camel in health and in disease.—382 pp. Stamford, Lincs., Haynes.
- LEWIS, D. J. (1947). General observations on mosquitos in relation to yellow fever in the Anglo-Egyptian Sudan.—Bull. ent. Res., **37**, pp. 543–566.
- LEWIS, D. J. (1948). The Simuliidae of the Anglo-Egyptian Sudan.—Trans. R. ent. Soc. Lond., **99**, pp. 475–496.
- LEWIS, D. J. (1949). The tsetse fly problem in the Anglo-Egyptian Sudan.—Sudan Notes, **30**, pp. 179–210.
- LLOYD, W. (1910). Notes on Kordofan Province.—Geogr. J., **35**, pp. 249–267.
- MACMICHAEL, H. A. (1922). A history of the Arabs in the Sudan. . . —**1**. Cambridge.
- MACMICHAEL, H. A. (1934). The Anglo-Egyptian Sudan. London. .
- MACMICHAEL, H. A. (1935). The coming of the Arabs to the Sudan.—In Hamilton, J. A. de C. Ed. The Anglo-Egyptian Sudan from within, pp. 40–60. London.

- MARCHAND, W. (1920). The early stages of Tabanidae (Horse-flies).—Monogr. Rockefeller Inst. med. Res., no. 13, 203 pp.
- MARTOGGIO, F. (1913). Sulle tripanosomiasi del dromedario critreo.—Ann. Igiene (sper.), (N.S.) **23**, pp. 229–234.
- MATHESON, R. (1950). Medical entomology.—2nd edn., 612 pp. New York, Comstock.
- MELLOR, J. E. M. (1932). Notes from Zanzibar, Tanganyika, Kenya, Uganda, and the Sudan : August to December, 1928.—Ent. mon. Mag. **68**, pp. 234–252.
- MORISON, C. G. T., HOYLE, A. C. & HOPE-SIMPSON, J. F. (1948). Tropical soil—vegetation catenas and mosaics. . . —J. Ecol., **36**, pp. 1–84.
- NEAVE, S. (1906). Report of travelling pathologist and naturalist.—2nd Rep. Wellcome trop. Res. Lab., pp. 183–204.
- NEAVE, S. A. (1915). The Tabanidae of southern Nyasaland with notes on their life-histories.—Bull. ent. Res., **5**, pp. 287–320.
- NIKOL'SKAYA, M. N. (1948). Species of the genus *Telenomus* (Hymenoptera, Scelionidae), parasites of the eggs of Tabanids. [In Russian.]—Dokl. Akad. Nauk SSSR, (N.S.) **62**, pp. 729–732. (R.A.E., (B) **39**, p. 161.)
- OLDROYD, H. (1952). The Horse-flies (Diptera : Tabanidae) of the Ethiopian Region. Vol. I. *Haematopota* & *Hippocentrum*.—London, Brit. Mus. (Nat. Hist.) 226 pp.
- PAUL, A. (1950). Notes on the Beni Amer.—Sudan Notes, **31**, pp. 223–245.
- PEEL, S. (1904). The binding of the Nile and the new Sudan. London.
- PHILIP, C. B. (1951). The Tabanidae (Horseflies) of Minnesota, with special reference to their biologies and taxonomy.—Tech. Bull. Minn. agric. Exp. Sta., no. 80, 132 pp.
- PHILIP, C. B. (1948). Notes on Egyptian Tabanidae with comment on certain supraspecific categories of Old World Tabanidae.—Bull. Soc. Fouad ler Ent., **32**, pp. 77–83.
- POSTIGLIONE, E. (1935). Il servizio veterinario e le più gravi malattie diffusibili del bestiame nelle nostre Colonie dell'Africa Orientale.—Clin. vet., Milano, **58**, pp. 614–711. (R.A.E., (B) **24**, p. 86.)
- PRICOLO, A., & FERRARO, G. (1918). Circa il tripanosoma del camello della Colonia Eritrea.—*Ibid.*, **41**, pp. 522–524.
- REID, J. A. (1930). Some notes on the tribes of the White Nile Province.—Sudan Notes, **13**, pp. 149–210.
- REID, J. A. (1935). The nomad Arab camel breeding tribes of the Sudan. . . — In Hamilton, J. A. de C. Ed. The Anglo-Egyptian Sudan from within, pp. 113–129. London.
- RICARDO, G. (1908). Descriptions of thirty new Species of Tabani from Africa and Madagascar.—Ann. Mag. nat Hist., (8) **1**, pp. 311–333.
- SANDON, H. (1951). The problems of fisheries in the area affected by the Equatorial Nile Project.—Sudan Notes, **32**, pp. 5–36.
- SCOTT, J. R. (1947). Kinana cattle.—Sudan Notes, **28**, pp. 181–183.
- SUDAN GOVERNMENT (1944). Report of the Soil Conservation Committee.
- SUDAN GOVERNMENT (1948). Report on the administration of the Sudan in 1946. Khartoum.
- SUDAN VETERINARY SERVICE. (1924–47). Annual reports for the years 1923 to 1946. Khartoum.

- SURCOUF, J. M. R. (1924). Les tabanides de France et des pays limitrophes.—Encycl. Ent., (A) **5**, pp. (261) Paris, Lechevalier.
- SURCOUF, J. M. R. & RICARDO, G. (1909). Etude monographique des Tabanides d'Afrique (groupe des *Tabanus*).—260 pp. Paris, Masson.
- TETLEY, H. (1918). The structure of the mouth-parts of *Pangonia longirostris* in relation to the probable feeding-habits of the species.—Bull. ent. Res., **8**, pp. 253–267.
- THEOBALD, F. V. (1904). Second report on economic zoology.—197 pp. London, Brit. Mus. (Nat. Hist.).
- TITHERINGTON, G. W. (1927). The Raik Dinka of Bahr el Ghazal Province.—Sudan Notes, **10**, pp. 159–209.
- TOTHILL, J. D. Ed. (1948). Agriculture in the Sudan.—974 pp. London, Oxford Univ. Pr.
- TWINN, C. R., HOCKING, B., McDUFFIE, W. C. & CROSS, H. F. (1948). A preliminary account of the biting flies at Churchill, Manitoba.—Canad. J. Res., (D) **26**, pp. 334–357.
- VAN SACEGHEM, R. (1916). Contribution à l'étude de la transmission du *Trypanosoma cazalboui*.—Bull. Soc. Path. exot., **9**, pp. 569–573.
- WENYON, C. M. (1908). Report of travelling pathologist and protozoologist.—3rd Rep. Wellcome trop. Res. Lab., pp. 121–168.
- WHITFIELD, F. G. S. (1939). Air transport, insects and disease.—Bull. ent. Res., **30**, pp. 365–442.
- WIEDEMANN, C. R. W. (1830). Aussereuropäische zweiflügelige Insekten, **2**.
- WIGGLESWORTH, V. B. (1950). The principles of insect physiology.—4th. edn., 544 pp. London, Methuen.
- WOODMAN, H. M. (1936). Filariasis.—Rep. Sudan med. Serv. 1935, pp. 67–68.
- WOODMAN, H. M. (1948). Filariasis in the southern Sudan.—E. Afr. med. J., **25**, pp. 95–104.
- WOODMAN, H. M. (1949). Filaria in the Anglo-Egyptian Sudan.—Trans. R. Soc. trop. Med. Hyg., **42**, pp. 543–558.
- WOODMAN, H. M. (1950). Filaria in the Sudan.—*Ibid.*, **43**, pp. 549–550.
- WOODMAN, H. M. & BOKHARI, A. (1941). Studies on *Loa loa* and the first report of *Wuchereria bancrofti* in the Sudan.—*Ibid.*, **35**, pp. 77–92.
- WORTHINGTON, E. B. (1946). Middle East Science.—239 pp. London, H.M.S.O.
- WORTHINGTON, E. B. (1950). Geography and the development of East Africa.—Geogr. J., **116**, pp. 29–43.
- ZUMPT, F. (1949). Medical and veterinary importance of horse-flies.—S. Afr. med. J. **23**, pp. 359–362.

RESPONSES OF PESTS TO FUMIGATION.

I. TOXICITY OF MERCURY VAPOUR TO THE EGGS OF *CALANDRA GRANARIA* (L.).*

E.M.N.

By R. E. BLACKITH and B. S. GORRINGE.

Imperial College Field Station, Sunninghill, Berks.

Insects and other pests respond to changes in the environment in ways which may be recognised and investigated quantitatively. Responses such as death, incoordination, paralysis and changes in gaseous exchange may result from alterations in temperature, pressure and composition of the atmosphere, etc. In a series of papers, of which this is the first, it is hoped to present some results of the study of these various factors and the associated responses. The experimental designs used permit detection not only of the influence of isolated environmental changes on certain responses but also of the mutual influence of multiple changes.

Mercury vapour has been used as an insecticide for small quantities of grain in underground stores in India for many years and observations of its ovicidal properties have been made in the laboratory from time to time. Wright (1944) and Richards (1945) have investigated the effect of mercury vapour on the eggs of various stored products pests with particular attention to *Calandra granaria* (L.) on wheat. In most of their experiments these workers endeavoured to ensure that eggs were exposed to an atmosphere saturated with mercury vapour; in some an unknown concentration gradient was established, but no measurements of concentration were made.

The work described here has been undertaken as part of an investigation in the possible use of mercury vapour as a fumigant. Eggs of *C. granaria* laid by insects obtained from various sources have been exposed to mercury vapour at known concentration \times time products and estimates made of the toxicity of the vapour to the different strains studied. One particular strain of insects bred in the laboratory has been found to develop resistance to mercury vapour, presumably associated with the accidental contamination of the incubator in which they were bred.

Methods of obtaining Eggs.

The technique employed was the same as that described by Richards (1945) for his studies on the effect of mercury vapour on the eggs of *C. granaria*. The breeding was carried out at 25°C. on English wheat of moisture content between 15 per cent. and 16 per cent.

A number of fertilised female weevils was put individually into 3 \times 1 in. glass tubes closed with corks bored with a $\frac{1}{2}$ in. diameter hole, the hole being closed with muslin. These females were then allowed to oviposit for six successive two-day periods on two grains of wheat each. The wheat grains were removed on alternate days and placed in 70 per cent. alcohol for 24 hours before dissection under a binocular microscope, and the number of eggs laid counted. The females were then arranged in three groups of twenty weevils, which had approximately equal oviposition scores in the 12-day testing period. The wheat grains cleared from each of these three groups on alternate days were ready for immediate use.

*This work forms part of a thesis by B. S. Gorrings (now at Research Laboratories, Kodak Ltd., Harrow), approved by the University of London for the degree of Ph.D.

Apparatus.

The apparatus employed for establishing known concentrations of mercury vapour was part of a detector used in studying the sorption of mercury vapour by wheat and has been described in detail elsewhere (Gorringe, 1950).

Glass reaction vessels, 1 litre in volume, were used as dosage chambers for the eggs. Known concentrations of mercury vapour in air were established in these vessels by evacuation and drawing in samples from a 5-litre mixing vessel fitted with provision for stirring a known quantity of mercury-saturated air with clean air.

The following procedure was adopted for obtaining known concentrations of mercury vapour in the dosage chambers. The mixing vessel and mercury-saturator system were assembled in a constant temperature enclosure. The mixing vessel was first evacuated and connected to a mercury manometer. Mercury-saturated air was then admitted from the saturator system to a pressure, measured on the manometer, that would give the required final concentration of mercury vapour. Clean air was admitted until atmospheric pressure was reached and the gases thoroughly mixed with a special stirrer. The concentration present in the vessel could then be calculated from the manometer readings knowing the saturation vapour pressure of mercury at the temperature of the enclosure.

After placing the wheat grains, containing the insect eggs to be treated, in the reaction vessel it was evacuated, connected to the mixing vessel, the pressures were allowed to equalise and finally the pressure in the reaction vessel brought to atmospheric by the addition of clean air. The final concentration of mercury vapour in the reaction vessel was then calculated from the relative volumes of the mixing and reaction vessels.

Preliminary Dosage-Response Curves.

Some experiments to determine the general features of the dosage-response curve were carried out in April and December 1948. In these experiments the concentration of mercury vapour was fixed as the saturation concentration at the temperature of the experiment and a graded series of responses obtained by varying the time of exposure of the eggs.

Preliminary experiments had shown that keeping eggs under vacuum for 5 to 10 minutes had no effect on the number subsequently hatching. In the first experiment (April 1948), two groups of 40 wheat grains, containing eggs, were placed in separate reaction vessels and each vessel evacuated before filling with air saturated with mercury at 20°C. This gave a concentration of 13.0 mg. mercury/m.³ in the vessels. A third, control, group was also evacuated and then allowed to fill with clean air. The vessels were placed in a constant temperature enclosure at 20°C. and allowed to stand for periods of up to 26 hours before being flushed out with clean air. The grains were replaced in their glass tubes and allowed to stand in the incubator for the remainder of the five-day incubation period before being soaked in 70 per cent. alcohol and dissected in the usual way to count the numbers of larvae and undeveloped eggs.

The second experiment, carried out in December 1948, was similar except that the concentration used was the saturation concentration of mercury vapour in air at 25°C. (20 mg./m.³) The experiments were carried out at 25°C., and the maximum exposure time was 72 hours. Probit regression lines were fitted to the data obtained from these two experiments by the method of Finney (1947) using a logarithmic normalising transformation of the dosage (concentration-time product in mg. hr./m.³) On this scale the median lethal doses and the slopes of the regression lines were calculated, the arithmetic values of the first parameter and the values of the latter on the logarithmic scale being shown in Table I.

The suggestion that the resistance of the insects was increasing with time, and the tacit assumption of the absence of a concentration-time interaction in these preliminary experiments seemed worthy of more extensive investigation. A relatively large-scale experiment was therefore designed to examine these points and to determine with some precision the characteristics of the dosage-response curve for the action of mercury vapour on the eggs of *C. granaria*.

Design of the Experiments.

Four groups of laying females, of different fecundity, were used to provide one batch of eggs three times each week. Forty eggs were then selected from each batch for the experiments. Since information was not available on the influence of fecundity on the tolerance of the eggs laid, it was decided to segregate the 52 egg batches used into 13 sets of four, laid over the same period, and 4 sets of 13, laid by each group. These sets are referred to subsequently as "groups" and "blocks" respectively.

More treatments (concentration \times time products) were desirable than "groups" were available. A Youden square variation of the balanced incomplete block design was adopted to meet this difficulty. The appropriate combinatorial solution available having equal numbers of blocks and treatments as required by the Youden square arrangement, accommodated 13 treatments. These consisted of the combinations of 3 concentrations and 4 times of exposure (12 concentration \times time products) together with controls consisting of eggs kept for the longest exposure period in the absence of mercury. This arrangement provided four complete replications of the experiments. The usual row, column and treatment randomisations were done in laying out the experimental design.

The concentrations used ranged from 1.59 mg./m.³ to 20 mg./m.³, and the exposure times from 4 hr. to 75 hr. The concentration \times time products were equally spaced, on a logarithmic scale, within these ranges.

Analysis of Data.

The analysis of the data was carried out according to a scheme of Fisher and Yates (1948). Since, in the event of equal variances for the inter- and intra-block comparisons, 18.8 per cent. of the available information lies in the former comparisons, it was decided to recover such information.

The data obtained in terms of percentage mortalities were converted by means of the angular transformation into quantities having equal weights, after adjustment for the number of eggs in each batch used, in the subsequent analysis of variance.

In this analysis the variance ratio for "groups" does not reach significance at the 5 per cent. level whereas that for "blocks" is a borderline case. The treatment effects are significant.

The corrected treatment means, adjusted for control responses, were transferred from angular units to probit units for subsequent analysis. Two sets of treatment means were used, one set (*a*) being based on the intra-block comparisons only, the other (*b*) derived from the total available information. Probit regression lines were fitted to both sets of data, the equation for the set (*a*) being $Y = 3.08 + 0.421x$. ($\chi^2_{11} = 38.23$, heterogeneous). The data from the total available information are fitted satisfactorily by the equation, $Y = 2.85 + 0.504x$. ($\chi^2_{11} = 10.90$ homogeneous) where x is \log_{10} (C.T. product).

Interpretation of the analysis.

Since the χ^2 residue for the more accurate regression line does not reach significance, there is no reason to add an additional term to the above equations, representing the C \times T interaction. Further if C \times T = constant, the *b* parameters of a probit plane, $Y = a + b_1 \log C + b_2 \log T$, would not differ significantly and the

fitting of such a plane would not provide any more information than the regression line given above.

Two conclusions may be drawn from this experiment, that a marked increase in the resistance of the eggs of the laboratory strain occurred, and that the slope of the regression line has decreased to an extent which is unusual in biological assays when the "availability" of the insecticide has not been altered by changing the physical condition of the assay.

A likely explanation for the increase in resistance is the development of a resistant strain of the weevils, caused by the presence of traces of mercury vapour in the incubator in which they were bred. Two considerations, however, required discussion. The first was the possibility that the "natural" variation of the test insect was so great that the values obtained could have arisen by chance. The second is that the change of slope of the regression line is in the opposite direction to that caused by truncating the normal distribution of tolerances by selection. Thompson (1950) has pointed out that the effect of truncation is to reduce the estimated standard deviation. The slope of a regression line would be increased if estimated from a population the tolerance distribution of which has been truncated, with the exclusion of the lower tolerance levels.

This effect would be expected if, as is often considered to be the case, the slope of the regression line is inversely proportional to the standard deviation of the distribution. On the other hand Stringer (1948) has related the slopes of pairs of regression lines to the relative availability of the insecticide in any two systems under comparison.

The availability coefficient (f) relates the weight (w) of insecticide acquired by the insect (or a suitable dose metameter) to the applied dosage (λ). There is often a tacit assumption of proportionality between these two quantities which is seldom justifiable. Stringer showed that the expression $w = \lambda^f$ is a more general representation of this relationship and that a change in f will preserve the linearity of a regression line but will alter its slope.

Further, Stringer demonstrated that in the particular case studied by him the ratio of slopes for two assays was the same as the ratio of the corresponding availability coefficients, i.e., $\frac{f_1}{f_2} = \frac{b_1}{b_2}$ and this relationship is probably of importance for many other systems as well. Now, if a resistant strain of the insects has emerged by selection in the presence of mercury vapour, one possible mechanism is that eggs with less permeable choria would survive. Such a selective process would reduce the availability coefficient and the slope of the regression line. The results reported are consistent with the suggestion that the permeability of the egg chorion to mercury vapour is a limiting factor in this assay system, masking truncation of the tolerance distribution. Since the slopes of the regression lines for the normal and resistant stocks are so different, their relative tolerances cannot be compared by analogy with relative potency assessments. It is, however, important to establish the significance of the increase in the median lethal dose for the laboratory strain of weevil eggs. The magnitude of the effect makes its chance occurrence unlikely but to settle this issue six populations were obtained, through the courtesy of the Infestation Division of the Ministry of Agriculture and Fisheries, from widely separated infestations of *C. granaria* not likely to have been at any time in contact with mercury vapour. The locations of the infestations from which the six populations were obtained are shown in Table I. The median lethal doses for each of the populations, together with the slopes of their regression lines, were estimated by the same technique as that described previously. Since in the earlier case the regression had been proved linear, and homogeneous, three dosages were thought sufficient, in these experiments. Advantage was taken of

TABLE I.
Comparison of regression parameters for unselected and resistant populations of *C. granaria*.

Source of Infestation	Locality of Collection	Collector	M.L.D. (log scale) (μ)	V (μ)	M.L.D. (linear scale) mg hr./m. ³	Slope (b)	V (b)
Warehouse ...	Basingstoke ...	H. P. Smith ...	—	—	< 1.0	—	—
Warehouse ...	London Docks ...	S. W. Yeomans ...	1.678	0.11	47.7	3.33	3.65
Warehouse ...	Bristol Docks ...	S. W. Bailey ...	1.740	0.04	55.0	2.65	1.74
Maize from Odessa	S.S. Alexandr	Miss E. H. Shaw	—	—	< 1.0	—	—
English Wheat in Barn	Suvorov (U.S.S.R.) Bere-Regis Dorset	D. J. Parry ...	1.043	0.13	11.0	3.71	2.08
Iraqi Barley ...	Alexandra Warehouse Gloucester Docks	D. J. Parry ...	1.261	0.98	18.3	1.50	0.52
Mercury resistant strain tested 21/4/48 – 15/5/48		...			180.7	1.46	1.50
Mercury resistant strain tested 8/12/48 – 14/1/49		...			356.0	1.61	0.85
Mercury resistant strain tested 11/2/49 – 18/3/49		...			18,510.0	0.50	0.0037

the absence of a concentration-time interaction to hold the concentration constant at 20 mg./m.³ while varying the times of exposure, the periods chosen being 3, 6 and 12 hours.

Fig. 1 shows that, in spite of the generally rather low precision of the estimates compared with those from the much larger experiment in balanced incomplete blocks, there is a clear distinction between the range of the parameters for the "wild" populations and those of the suspected resistant strain. The two parameters for the latter are in each case significantly different from the corresponding estimates from the wild populations, the median lethal dose being higher, the slope lower.

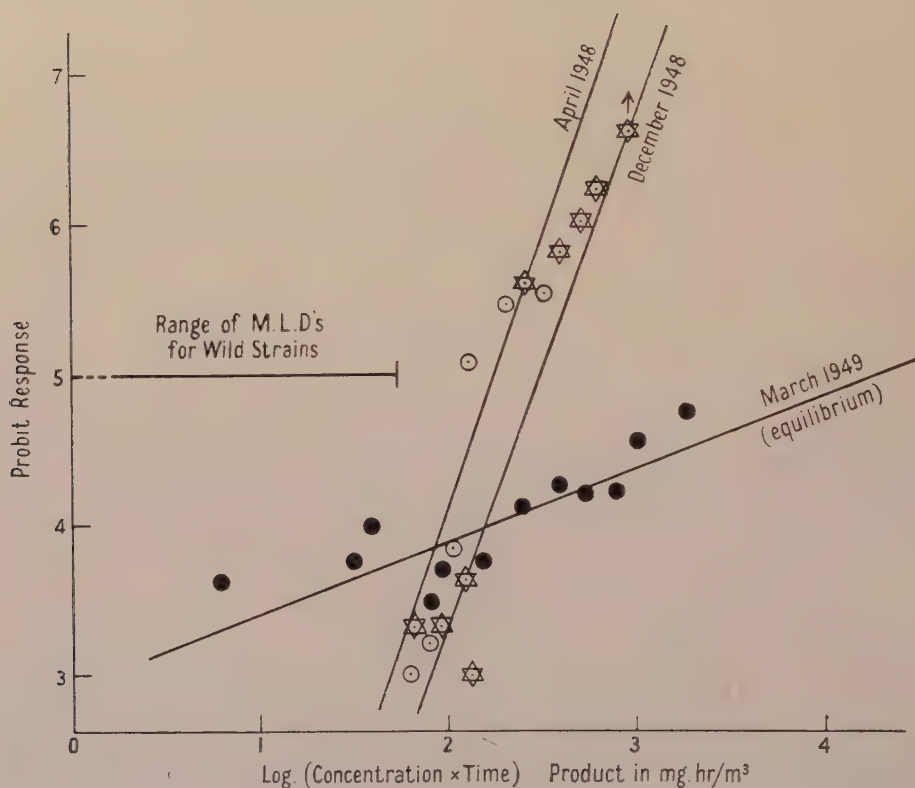


Fig. 1.—Variation of resistance of laboratory strain of *Calandra granaria* with time.

The saturation concentration of mercury vapour in air is 20 mg./m.³, at 25°C. and the eggs normally hatch at this temperature in 5 days, giving a concentration-time product of 2,400 mg.hr./m.³. It is a feature of the resistant strain that within the limits imposed by the saturation vapour pressure of mercury and the normal duration of the egg stage of the insect at any particular temperature, no concentration-time product can be attained which will kill more than half the eggs of this resistant strain of grain weevils. For this strain the log. median lethal dose is 4.267 with 95 per cent. fiducial limits 4.006 and 4.755. The corresponding arithmetic values are 18,510 for the median lethal dose, with limits at 56,950 and 10,005, in units of mg.hr./m.³. The ratios of the median lethal doses indicate that concentration-time products several thousand times as great are needed to kill the resistant strain compared with wild populations.

The rapid development of resistance, which so far as can be judged attained its limiting value in less than ten generations, suggests that mercury-resistant strains of *C. granaria* may arise fairly easily in the field.

If the change in the slopes of the regression lines is related solely to a change in the availability coefficient, then the resistant strain has a chorion permeability about one fifth of its average value in unselected populations.

Against this disadvantage can be set the toxicity of mercury vapour to the eggs of *C. granaria*, which is generally of a higher order than that of most fumigants to the eggs of stored products insects.

Effect of Mercury Vapour on gravid Females.

Eggs retained by adult females might also be killed by the fumigant, so that this point was investigated in the following way. A batch of 30 weevils was withdrawn from five of the six "wild" populations listed in Table I, and, after sexing and a two-day recovery period on clean wheat, 15 of these insects were submitted to the highest concentration-time product used in the experiments on wild eggs, namely 240 mg.hr./m.³. The remaining 15 insects were retained as controls. Both sets of insects were placed on clean wheat and allowed to oviposit for two days, the wheat grains containing the eggs were removed, and, after the usual two-day incubation period, dissected.

Table II shows the numbers of fertile and infertile eggs obtained from the "fumigated" and "control" sets of females for each population. In none of the five cases was there any significant reduction of egg viability caused by the exposure of the gravid females to mercury vapour.

TABLE II.
Exposure of gravid females to mercury vapour.

Source of Population	Treatment	No. of eggs		χ^2_1
		Hatched	Not Hatched	
Iraqi Barley	Fumigated	5	6	} No reduction of viability
	Control	6	9	
Dorset Wheat	Fumigated	8	16	} 2.106
	Control	18	16	
Bristol Wheat	Fumigated	10	29	} 0.502
	Control	8	13	
Basingstoke Wheat	Fumigated	1	30	} No reduction of viability
	Control	0	22	
Odessa Maize	Fumigated	2	26	} No reduction of viability
	Control	5	31	

The upper 5% point for χ^2 with 1 d.f. is 3.84.

Treatment of Grain before Oviposition.

Richards (1945) showed that treatment of grain with saturated mercury vapour for 20 days did not affect eggs subsequently laid in the grains. This investigation was extended to cover a much longer period of exposure of the grain to the fumigant.

Grain of moisture content between 15 per cent. and 16 per cent. was enclosed in an airtight desiccator with an open and clean mercury surface for two years. A sample

was then withdrawn, and 10 grains were placed in a 3×1 in. tube, together with 10 of a sample of 20 female *C. granaria* which had been allowed to recover from the sexing process on clean wheat. The remainder of the females were placed on 10 grains of clean (unfumigated) wheat of the same moisture content.

After the usual period had been allowed for oviposition, and for incubation, the grains were dissected. In the fumigated grain 14 eggs proved to be fertile and 24 sterile; in the clean grain 8 were fertile and 24 sterile. It cannot, therefore, be said that the mercury vapour, even after the prolonged contact, has any toxic action as a residue in the grain.

Summary.

Wild populations of *Calandra granaria* were allowed to oviposit on clean wheat and the tolerances of the eggs to mercury vapour were estimated. For six such populations the median lethal doses ranged from 55 mg.hr./m.³ to less than 1.0 mg.hr./m.³. A laboratory strain of grain weevils has developed resistance to mercury vapour in the egg stage, probably from contamination of an incubator. Such evidence as is available suggests that this resistance is associated with reduced chorion permeability rather than enhanced metabolic ability. The increase in resistance is such that no attainable concentration \times time product will kill more than half the resistant egg population which is thus virtually in equilibrium with air saturated with mercury vapour. Fumigation of gravid females, or of wheat prior to oviposition, does not reduce the viability of any eggs subsequently laid.

Acknowledgements.

The authors are greatly indebted to the officers of the Infestation Division, Ministry of Agriculture and Fisheries, who are listed in Table I, for their help in collecting the wild strains used in these experiments. Much of the tedious and exacting work of dissecting the wheat grains was done by Miss B. Drew.

This work was carried out by means of a grant from the Agricultural Research Council, whose interest and assistance are hereby acknowledged.

References.

- FINNEY, D. J. (1947). Probit analysis. . . .—256 pp. London, Cambridge Univ. Press.
- FISHER, R. A. & YATES, F. (1948). Statistical tables for biological, agricultural and medical research.—3rd edn. London & Edinburgh, Oliver & Boyd.
- GORRINGE, B. S. (1950). Determination of fumigants. XXI. Preliminary experiments on the sorption of mercury vapour by wheat.—J. Sci. Fd. Agric., **1**, pp. 114–118.
- RICHARDS, O. W. (1945). The effect of mercury vapour on the eggs of *Calandra granaria* (L.) (Col., Curculionidae).—Bull. ent. Res., **36**, pp. 283–290.
- STRINGER, A. (1948). Relation between bioassay systems and the values found for toxicity of DDT.—Ann. appl. Biol., **35**, pp. 527–531.
- THOMPSON, H. R. (1950). Truncated normal distributions.—Nature, **165**, pp. 444–445.
- WRIGHT, D. W. (1944). Mercury as a control for stored grain pests.—Bull. ent. Res., **35**, pp. 143–160.
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RESPONSES OF PESTS TO FUMIGATION.

II. TOXICITY OF HYDROGEN CYANIDE TO *CALANDRA* SPP. UNDER REDUCED PRESSURE.*

By K. F. SALMOND.

Imperial College Field Station, Sunninghill, Berks.

In the present work, which was of a preliminary nature, the insects used were adults of *Calandra granaria* (L.) and *C. oryzae* (L.) which had been separated into sexes. The main criterion of response was death and a subsidiary one was oviposition by females surviving fumigation. The environmental factors were pressure, concentration of hydrogen cyanide, period of exposure and removal from food for a period before treatment. There were two independent sets of experiments in one of which a reduced pressure was maintained throughout the period of exposure :—fumigation with sustained pressure reduction. In the other method air was added, together with the fumigant, so as to re-establish atmospheric pressure around the insects :—fumigation with preliminary pressure reduction.

Material and Experimental Methods.*Insects.*

The weevils were bred on good quality English wheat which had been fumigated with methyl bromide to kill any adventitious insects which might have been present but which, however, subsequent examination failed to reveal. Only whole grains were used in the experiments on fumigation or on egg-laying.

Wheat for experiments on fumigation or on egg-laying was freed from broken grains and dust and conditioned in an incubator at 27°C. and 60 per cent. relative humidity for one week; *C. granaria* was bred at 25°C. and 70 per cent. relative humidity and *C. oryzae* at 27°C. and 60 per cent. relative humidity. Freshly emerged adults, of either species, were kept on fresh wheat for a period of from three to four weeks, which was short enough to avoid risk of emergence of weevils from a fresh generation. Grains were dissected for the counting of larvae and eggs, and weevils were separated into sexes by a method similar to that of Richards (1947). It was found convenient to expose the last abdominal segment of *C. granaria* by placing insects on their backs on the microscope stage and pressing the abdomen gently with the left fore-finger.

Weevils for treatment were contained in glass tubing, $\frac{1}{2}$ in. diameter, closed with muslin secured with "cellotape". Each tube contained 15 weevils. Tubes with fed insects and with those starved for 24 hours of both sexes and both species, making eight tubes in all, were used for each treatment. Tubes of fed insects contained eight grains of wheat while tubes with unfed insects contained, instead, a piece of filter paper to support the insects.

Fumigant.

The sample of hydrogen cyanide used was obtained by distillation of the commercial liquid in an all-glass, "pyrex", apparatus and stored in a "pyrex" bottle in a refrigerator. All-glass "pyrex" vessels of known capacity, *circa* 1 litre, of the type described by Turtle (1941), were used as fumigation chambers.

*Part of a thesis approved by the University of London for the degree of Ph.D.

The gas was introduced by breaking, in the closed chamber, a marked ampoule containing a weighed amount of fumigant, previously introduced into the ampoule from a burette with a very fine jet fixed to the tip. The filling was done in a refrigerator, the ampoules being kept in ice-water until sealed off with a small flame (*cf.* Lubatti, 1935). The period of exposure of the insects to the fumigant was considered to begin when the ampoule was broken and to end when the tubes of insects were withdrawn from the chamber. The tubes of insects were placed on wire gauze trays and stored in an incubator at 27°C. and 60 per cent. relative humidity for 72 hours after which mortality was determined by spreading the insects on a white enamel tray and warming under a lamp. Starved insects were given grain during the necessary period.

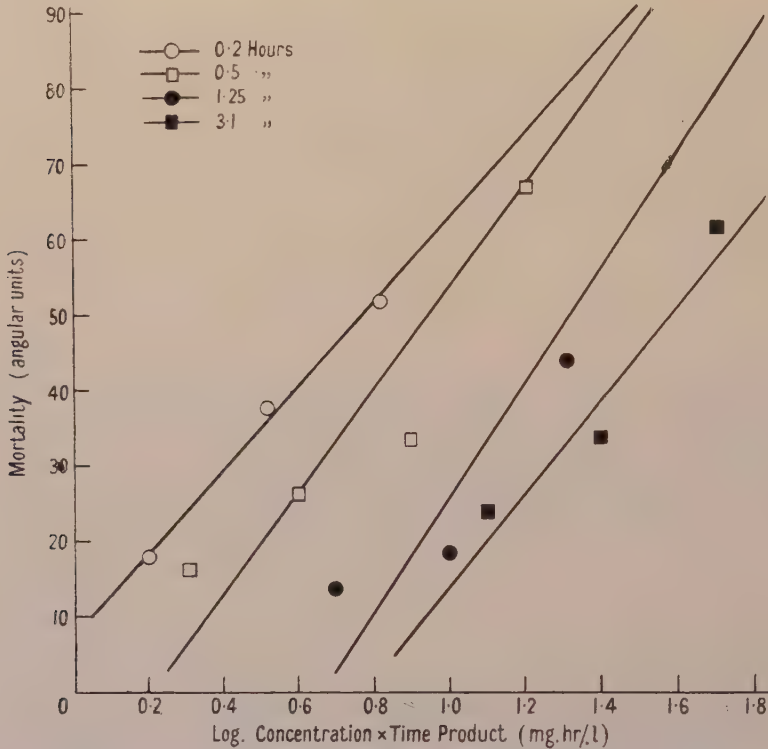


Fig. 1.—Mortality of *Calandra* spp. fumigated with preliminary pressure reduction.

The concentration of fumigant was taken as the nominal concentration, derived from the dose and the capacity of the chamber, the assumption being that losses by sorption were small, independent of the factors studied and unlikely, if allowed for, to modify the conclusions drawn from the analysis. A simplification of the analysis of results was thereby secured and this is considered to be sufficient justification of the procedure in this preliminary investigation. For similar reasons consideration of humidity relations during fumigation is reserved for a later study.

The dosage operations in the two methods of fumigation are as follows :—

(a) Sustained pressure reduction.

Pressure in the chamber containing insects was reduced for two minutes to 15 mm. of mercury, and then raised to atmospheric with dried air. Immediately after filling

with dry air, the pressure was reduced to the required value and the ampoule was broken by shaking, whilst the chamber was rocked to assist distribution. It was then placed in the incubator.

(b) Preliminary pressure reduction.

As with method (a) but after breaking the ampoule the pressure was finally raised to atmospheric by admitting dried air and allowed to remain at that level for the duration of the experiment.

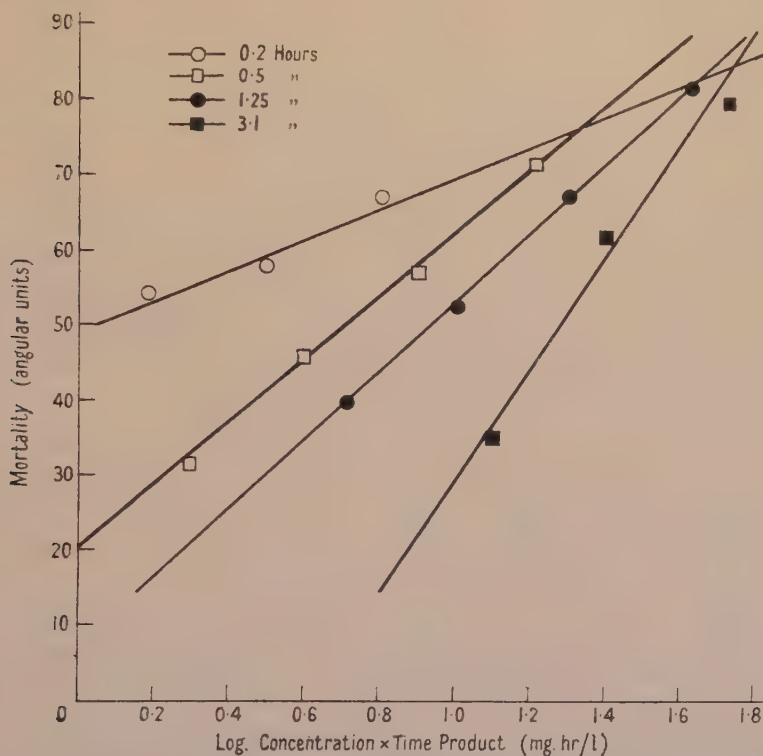


Fig. 2.—Mortality of *Calandra* spp. fumigated with sustained pressure reduction.

Experimental design.

The design was a balanced incomplete block arrangement of the type known as a Youden square. Only seven experiments could be performed on any one occasion of testing and the first requisite was the possibility of measuring the variability of the insects between occasions. In the design adopted, there were 15 occasions of testing, or experimental blocks. The same number of concentration-time products were applied and by a further restriction a complete set of such treatments appeared in each of the seven replications specified by the design. Comparisons between replicates are orthogonal to all other comparisons and this arrangement enabled the additional factor to be introduced at the same number of levels as there are replicates, *viz.*, seven, thus providing for the inclusion of seven different pressures. Of the 15 treatment levels, 14 were allotted to concentration-time products, equally spaced on a logarithmic scale between 1.6 and 49.6 mg. hours per litre and one to a treatment at zero concentration and the longest exposure period, 3.1 hours. Pressures were equally spaced, on a logarithmic scale, between 2 and 76 cms.

Interpretation of data.

The percentage responses for each batch of insects were converted, by means of the angular transformation, into quantities to which an analysis of variance was applied. Those factors and their interactions which this analysis disclosed as important were examined further by plotting the transformed responses against the logarithms of the concentration-time products (figs. 1-4).

Results and Discussion.*Food and sex.*

Neither of these factors influenced the mortality of either species of *Calandra* nor were there any interactions with the other factors investigated. According to Gough (1939), *Tribolium confusum* Duv. behaves differently, the females being more susceptible to atmospheric fumigation with hydrogen cyanide than the males.

Treatments with fumigant.

The usual relationship in which logarithmic increments of concentration and of time produce additive increments of mortality, Haber's Rule, requires modification, according to the present results.

Figs. 1 and 2 show that, both for sustained and for preliminary pressure reduction, short periods of exposure and high concentrations give consistently higher responses than do the same concentration-time products made up of long exposures and low

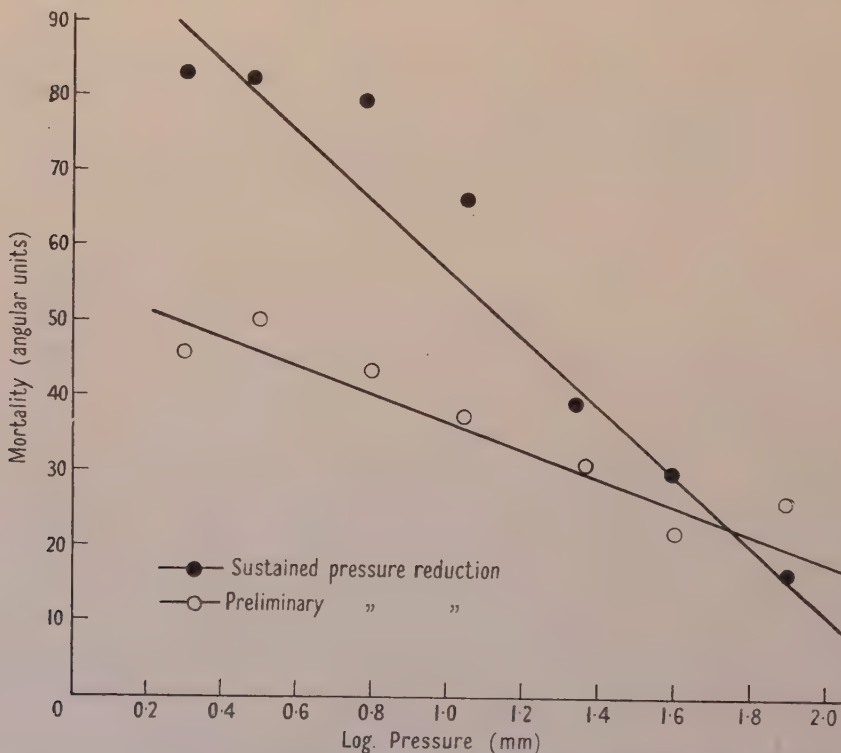


Fig. 3.—Mortality of *Calandra* spp. for fumigations with sustained and with preliminary pressure reduction.

concentrations. Further, for preliminary pressure reduction, the rate of increase of mortality for a given increase in dosage (measured by the concentration-time product) is significantly reduced when short exposure periods are employed, and this relation is consistent with a reduced availability of the fumigant to the insect.

Pressures.

Throughout these experiments preliminary pressure reduction has much less effect on mortality after fumigation than has sustained pressure reduction but both methods give higher mortalities than those given without pressure reduction (see fig. 3). The statistical analysis discloses that with preliminary pressure reduction the

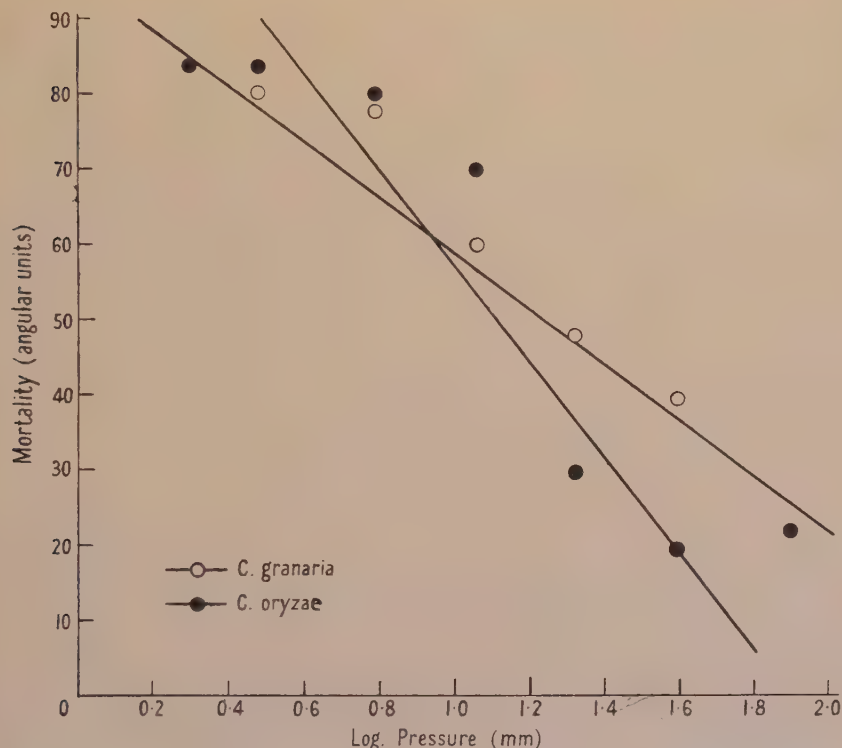


Fig. 4.—Mortality of *Calandra granaria* and *C. oryzae* for fumigations with sustained and with preliminary pressure reduction.

mortalities of *C. granaria* and *C. oryzae* are about the same but with sustained pressure reduction the mortality of *C. oryzae* is higher than that of *C. granaria* and the increase in mortality for a given increase in pressure reduction is also greater for *C. oryzae* than for *C. granaria*. In order to simplify the presentation, however, figs. 3 and 4 show the responses of the two species averaged over the two methods of fumigation.

However, under these experimental conditions, pressure reduction without fumigation increases mortality in control batches of *C. oryzae* much more than in those of *C. granaria*. Such increases account for a large part of the increased susceptibility observed when pressure reduction is accompanied by fumigation.

The increase of susceptibility of insects, particularly *C. oryzae*, which accompanies reduction of pressure is known to be closely associated with concomitant loss of

water which itself is regulated partly by atmospheric humidity. This subject will be further examined in later papers in this series.

Oviposition.

The number of eggs laid by females surviving the treatment was estimated. None of the factors investigated could be discriminated, using this response, from the excessive variability of the number of eggs laid both by treated and untreated females. Excessive variation in egg number seems general for several species of insects.

Summary.

Adults of *Calandra granaria* and *C. oryzae* have been fumigated with hydrogen cyanide by the methods of sustained pressure reduction and preliminary pressure reduction, the main criterion of response being death. Of the factors studied, starvation and sex of the insects are without influence. With pressure reduction, Haber's Rule relating mortality, concentration and period of exposure is not followed; for a given concentration-time product, the mortality is higher, the shorter the period of exposure.

Preliminary pressure reduction increases mortality much less than sustained pressure reduction. With the latter the mortality of *C. oryzae* is higher than that of *C. granaria*. The increases in susceptibility are largely accounted for by increases caused by pressure reduction alone in the absence of fumigant. The number of eggs laid by untreated females and by females surviving treatment was excessively variable and no discrimination between factors could be made.

Acknowledgements.

I am indebted to Professor J. W. Munro, C.B.E., for permission to carry out this work at the Imperial College Field Station.

References.

- GOUGH, H. C. (1939). Factors affecting the resistance of the flour beetle, *Tribolium confusum* Duv., to hydrogen cyanide—Ann. appl. Biol., **26**, pp. 533–571.
- LUBATTI, O. F. (1935). Determination of fumigants. III. Micro-determination of ethylene oxide and hydrogen cyanide—J. Soc. chem. Ind., **54**, pp. 424T–426T.
- RICHARDS, O. W. (1947). Observations on grain-weevils, *Calandra* (Col., Curculionidae). I. General biology and oviposition.—Proc. zool. Soc. Lond., **117**, pp. 1–43.
- TURTLE, E. E. (1941). Thesis, Univ. Lond.
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EXPERIMENTS ON THE EFFECT OF RESIDUAL INSECTICIDES IN HOUSES AGAINST *ANOPHELES GAMBIAE* AND *A. FUNESTUS*.

By G. DAVIDSON, B.Sc.

Colonial Insecticide Research Unit, Taveta, Kenya.

(PLATE V.)

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The experiments described in this paper were designed to confirm or otherwise the results recorded by Muirhead Thomson (1950) on the behaviour of *Anopheles gambiae* Giles and *A. funestus* Giles in huts treated with certain formulations of DDT and BHC (benzene hexachloride), and to elaborate his observations by including newer formulations. Using simple mud and thatch huts fitted with window traps, Muirhead Thomson (1950) showed that the majority of the females of *A. gambiae* that entered a hut treated with the DDT wettable powder, "Ditreen", escaped into the window trap without acquiring a lethal dose of the insecticide, while in a hut treated with the BHC wettable powder, P.530, most of the *A. gambiae* were killed inside the hut.

The present experiments, like Muirhead Thomson's, involved the construction of native-type huts, their occupation by African volunteers, and observation of the effect of spraying the internal surfaces of the huts with various insecticides on the mosquitos entering them. As in Muirhead Thomson's experiments, each hut was more or less light-tight except for a one-foot square opening, into which fitted an exit window trap, to catch any mosquitos attempting to leave the hut (Pl. V, figs. 2, 4).

The experiments were carried out at Taveta, Kenya, between February, 1950 and August, 1951, in an isolated forest area associated with permanent spring water in an otherwise open dry region. The rainfall for the year 1950 was 25 inches. The predominant mosquitos were *A. gambiae* and *A. funestus*, the latter present all the year round, the former occurring in large numbers during the main rainy season from March to June. The two Culicines, *Mansonia* (*Mansonioides*) *africana* (Theo.) and *Culex fatigans* Wied. were also common. Other species of Anophelines and Culicines were comparatively rare.

Three groups of experiments were carried out at three different periods using different formulations of different insecticides and three types of huts. These huts were all of the same dimensions : ten feet square with walls six feet high, and a sloping roof, but they differed in the materials used for their construction.

Group I.—The first experiments were an attempt to repeat as closely as possible Muirhead Thomson's experiments, using the BHC wettable powder, P.530, and the DDT wettable powder, "Ditreen", and including another DDT formulation—a DDT oil-bound suspension, "Supona" D (TP 724). The four huts (one acting as control) used in these experiments had walls made of the local earth and roofs of grass (Pl. V, figs. 1, 2). No special floors were constructed and no attempt was made to prevent ants from entering. The wall surface was hard and smooth but markedly cracked.

Group II.—In an attempt to produce a smooth uncracked wall surface, a further three huts were constructed, using a mixture of gravel and dung in equal parts for the walls. Although the cracking was less marked, the surface was comparatively soft and less smooth. In addition concrete floors were installed as a precaution against ants. These three huts were sprayed with two formulations of BHC and one of DDT, *viz.*, Murphy paste. Unintentionally the dosages of these insecticides, as determined by chemical analyses of sample papers put up at the time of spraying, were much lower than in other experiments.

Group III.—In the final series of experiments, the six huts sprayed in the previous two groups were completely relined internally and re-roofed, and a further three huts erected. All the huts had walls of a mixture of equal parts of the local earth and gravel, which gave a very hard smooth surface with only narrow cracks. The grass roofs were replaced by roofs of palm-thatch with a greater slope than previously, a precaution against the entry of rain. All the huts had concrete floors and four had concrete canals encircling them completely outside, which were kept permanently filled with water to eliminate ants (Pl. V, figs. 3, 4). It was originally intended to construct these canals around all the huts but a shortage of cement in the area prevented this. It so happened that little indication of the presence of ants was noticed throughout the observations made in these huts. The insecticides used were the two formulations of BHC used in the second group of experiments (but at a higher dosage), five formulations of DDT, including Murphy paste (but again in higher concentration), a mixture of BHC and DDT, and the new insecticide dieldrin. The one main control hut, constructed with the three huts used in the first group of experiments, remained the same, *viz.*, with a wall of the local earth and a roof of grass, throughout the three groups of experiments except for the addition of a concrete floor for the last group.

Methods of Collecting.

Mosquitos were collected from the experimental huts by the following methods :—

Hand catches.—Mosquitos remaining inside the huts after daybreak were collected between 7 and 9 a.m. with sucking tubes. Small nets were used to capture flying mosquitos which were common in the treated huts. The doors of huts were left open while hand catching and floor catching were being carried out in order to obtain

additional light ; and—to prevent any flying mosquitos from escaping—screened doors were fitted on the outside of the ordinary doors. Catches by hand were made daily in the first two groups of experiments, but in the third group this was done only once a week, because it was evident that any mosquitos remaining in the huts after daybreak would eventually be killed and collected in the floor searches, or, if they survived, would be caught later in the window traps.

Floor catches.—The floors were searched twice daily (7 a.m. and 2 p.m.) for the presence of dead mosquitos. In the first series of experiments the floors were covered with white sheets, but where concrete floors were installed it was found that dead mosquitos were quite easily recognised on this surface. The floors were swept after the afternoon catch each day.

Window trap catches.—Mosquitos attempting to leave the huts were collected with the aid of window traps of the usual "lobster-pot" type. Two of these traps were employed on each hut :—

- (a) *The evening window trap* to collect the mosquitos leaving at dusk. This trap was removed just after dark and replaced by :
- (b) *The morning window trap* to catch mosquitos leaving the hut during the night and early morning. This trap was removed after the hand and floor catching had been completed in the morning and, in later experiments, the opening into which the trap fitted was closed with a piece of cardboard to prevent any mosquitos still left alive in the hut from escaping through it before evening.

Mortality Observations.

Mosquitos caught alive by the various methods outlined above were transferred into small cages and the mortalities among them determined after varying intervals. In the original experiments these mosquitos were kept as long as 48 hours but, even after 24 hours, the mortalities among mosquitos collected from the control hut, especially unfed ones, were found to be very high. The provision of a diet of sugar or currants only improved the situation slightly. In the control hut it was found that in the case of mosquitos collected by hand in the morning and in the morning window trap, no significant difference was apparent between mortalities at 9 a.m. and at 2 p.m., but a significant difference did occur between the afternoon mortality and that of the following morning. Thus in compiling the results in this paper, the mortalities among mosquitos caught by hand and in the morning window trap are those recorded on the afternoon of the same day as the catch, *i.e.*, seven hours after hand catching and after the removal of the morning window trap. Some of the mosquitos may have entered the morning window trap anything up to 12 hours before its removal but, if, as is indicated by the observations of Haddow (1942), Muirhead Thomson (1948) and Hadaway (1950) on the habits of *A. gambiae* and *A. funestus* in Africa, the peak of entry into the hut is just before dawn, then it is probable that most of the mosquitos in the morning window trap will have been there only one or two hours before the trap was removed, or possibly two or three hours more if the peak of entry of *A. gambiae* is between 1 and 4 a.m. as suggested by Hocking and MacInnes (1948), whose observations were actually made at Taveta. As will be shown elsewhere, most of the mosquitos in the morning window trap in the control hut were unfed. As very few unfed mosquitos were found resting inside the hut during the day, it is concluded that those mosquitos which enter the hut do so in order to feed and not to seek shelter for the day as suggested by Haddow (1942), and leave for outside resting places if they have been unable to feed. In treated huts, some of the mosquitos in the morning window trap were freshly fed and these had presumably had contact with the insecticide and had been irritated and tried to leave the hut. Thus it seems reasonable to assume that most of the mosquitos in the morning window trap entered it shortly after entering the hut and, by the same afternoon, were less than 12 hours out of the hut.

The mortalities among the mosquitos collected in the evening window trap are those recorded in the morning of the following day, *i.e.*, about 12 hours after leaving the hut.

Other Experimental Observations.

In addition to the routine catches from the experimental huts and the recording of mortalities among them, three other series of observations were carried on at the same time, using wild-caught blooded female *A. gambiae* and *A. funestus* collected from native houses in the forest area.

Wall application tests.—Using the method described by the author (1950), *A. gambiae* and *A. funestus* were applied under petri dishes to the walls of the treated huts at regular intervals after treatments and for varying lengths of time. Mortalities 24 hours after their application were then recorded.

Tests for the fumigant and particulate effect of the insecticides.—*A. gambiae* and *A. funestus* were suspended in small cages inside the huts during the third group of experiments, without actual contact with the insecticidal surfaces, at frequent intervals and for varying lengths of time, and the mortalities among them recorded 24 hours after their removal.

Two-Cage tests.—To determine the normal resting time of *A. gambiae* and *A. funestus* on surfaces treated with various insecticides, before irritation and flying towards light is induced, a special apparatus was constructed—to be described later. The mosquitos so irritated were caught and the mortalities determined 24 hours after their removal to see whether the contact time with the insecticide was enough for them to acquire a lethal dose.

Contamination of apparatus.

Little attention was paid to the chances of the window traps, cages, etc., used in the first two groups of experiments becoming contaminated by the insecticides, and apart from occasional washings of the cages in soap and water no cleaning was performed. That there was some contamination however, is indicated by the results obtained in the controls in these experiments (see appropriate Tables). In the third group, however, the window traps and cages were dipped daily in a large bath of cleaning spirit (benzol) and frequently tested for contamination by leaving wild-caught female *A. gambiae* and *A. funestus* in them for 24 hours. There was little indication of contamination.

Application of insecticides.

The spraying of the insecticides was done with a Four Oaks pneumatic spraying machine. At the time of spraying, sample papers (blotting paper pads four inches square) were put up at random on the walls and under the roofs of the huts and later analysed* to determine the dosage applied. In addition, in the final group of experiments, small mud blocks of the same materials used in the making of the walls of the huts were distributed around the eaves of the huts and sprayed at the same time as the huts. At frequent intervals after treatment one of these mud blocks from each hut was analysed chemically to find the dosage of insecticide remaining on the surface and the proportion which had been absorbed inside the block.

Normal Behaviour of Mosquitos in untreated occupied Huts with Window Traps.

From catches made by the methods already described in four huts before any spraying was done (Tables I and II) and in the control hut after insecticidal treatments

*All chemical analyses of BHC and DDT deposits were made by the Chemical Department, Colonial Insecticide Research Unit, Arusha, Tanganyika.

of the other huts were carried out (Table XI), the following facts concerning the normal behaviour of *A. gambiae* and *A. funestus* emerge :—

Female A. gambiae.

1. The detailed figures from which Table I has been compiled show that of those leaving the hut more do so in the evening than during the night and early morning when these mosquitos occur in small or moderate numbers, but that although the overall average for these months (March to May) shows an excess of the evening catch over the morning catch, the trend for the period of peak production from April to June is in the reverse direction, as will be seen from the combined figures for *A. gambiae* and *A. funestus* given in Table XI where months 2 to 4 approximately correspond to this season of peak production.

2. Of those resting in the hut during the day, almost all have fed.
3. Of those leaving the hut in the evening, 59 per cent. are completely gravid.
4. Of those leaving the hut during the night and early morning most are unfed.

TABLE I.

Average daily catches of *A. gambiae* and *A. funestus* in four occupied experimental huts before their treatments with insecticides (March to May, 1950).

Method of catching	No. of daily catches	Females		Males	
		<i>A. gambiae</i>	<i>A. funestus</i>	<i>A. gambiae</i>	<i>A. funestus</i>
Daytime hand catch ...	129	46.8	7.4	0.8	1.9
Evening window trap ...	165	12.1	6.1	1.3	9.0
Morning window trap ...	164	9.3	2.2	0.01	0.1

TABLE II.

Stomach and ovary conditions among female *A. gambiae* and *A. funestus* caught in four occupied experimental huts before their treatments with insecticides (March to May, 1950).

Method of catching	Total mosquitos examined	Percentages			
		Unfed	Incompletely fed	Fed and partly gravid	Fully gravid
		<i>A. gambiae</i>			
Daytime hand catch ...	1,680	1	9	87	3
Evening window trap ...	1,053	17	13	11	59
Morning window trap ...	717	78	11	8	3
		<i>A. funestus</i>			
Daytime hand catch ...	337	9	17	68	6
Evening window trap ...	433	41	17	5	37
Morning window trap ...	183	82	11	4	3

Female A. funestus.

1. The same remarks apply here as under *A. gambiae* (1).
2. Of those resting in the hut during the day, most have fed, but the proportion of unfed or incompletely fed individuals is greater than in the case of *A. gambiae*.
3. Of those leaving at dusk, less than 40 per cent. are fully gravid, while 41 per cent. are unfed.

4. Of those leaving the hut during the night and early morning, most are unfed as in the case of *A. gambiae*.

It thus appears that fed females of both species stay inside the huts to mature their ovaries and when gravid leave at dusk ; very few unfed females rest in the huts during the day. The unfed individuals in the evening window trap were presumably those that entered the hut at dusk (when the huts were invariably unoccupied) and, finding nothing to feed on, left again. The unfed ones in the morning window trap were those that entered during the night and early morning and for some reason did not feed. During the peak season, when something like 1,000 mosquitos may enter one hut, one is led to the conclusion that only a small proportion of them are able to feed, and that this accounts for the large numbers occurring in the morning window trap during this period. A significant proportion of both species feed incompletely, *i.e.*, take only a small quantity of blood which is digested without any accompanying development of the ovaries. Possibly these individuals are very young unfertilised females ; they sometimes stay in the hut or sometimes leave with their blood undigested.

The figures in Table II indicate that a greater proportion of the *A. funestus* females collected from the huts and window traps were unfed or incompletely fed than of *A. gambiae* females. This would explain the fact that a greater proportion of the *A. funestus* females were found leaving the huts than resting in them during the day-time. Thus the fact that *A. funestus* is a less completely house-haunting species than *A. gambiae* would appear to be because it feeds less readily.

Both species showed a marked preference for resting on the roof during the day-time rather than on the walls or under beds. Of a total of 7,342 mosquitos (mainly *A. gambiae* and *A. funestus* females) caught by hand during the day in the four occupied huts before their treatments with insecticides (huts with grass roofs and mud walls), 6,527 were found on the roof, 622 on the walls and 193 under beds. Additional light from the window-trap opening was probably the cause of this distribution of the mosquitos. In an ordinary native hut without windows the distribution is probably more uniform.

Results of Treatments of Experimental Huts.

Group I Experiments (Tables III & IV, graph 1).

Three huts were sprayed at the end of May, 1950, with the following insecticides:—

BHC water-dispersible powder, P.530 (a mixture of BHC and goulac), containing 44 per cent. BHC, at an average dosage of 153 mg. BHC (20 mg. of the gamma isomer) per square foot.

DDT wettable powder, "Ditreen," containing 37.7 per cent. DDT, at an average dosage of 98 mg. DDT per square foot. "Ditreen" and "Supona" are registered trademarks of Shell Petroleum Co. Ltd.

DDT oil-bound suspension "Supona" D, containing 43.5 per cent. DDT, at an average dosage of 309 mg. DDT per square foot.

Spraying was done towards the end of the mosquito season and the results obtained during the second to the fifth months after treatment are based on very small numbers of mosquitos. Observations were carried on for nine months to determine when the effect of the insecticides had completely disappeared.

BHC P.530 hut.

Almost complete kills were obtained in this hut in the first two months after treatment, less than one-third of the mosquitos escaping even as far as the window traps. Even in the third and fourth months, over 50 per cent. kills of female

TABLE III.

Group I Experiments.

Monthly analysis of hut catches and mortalities among female *A. gambiae* and *A. funestus*.

Months after treatment	Total mosquitos	Percentage distribution of mosquitos			Overall percent- age mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		*Inside hut	Evening window trap	Morning window trap	
BHC P.530 (153 mg. BHC per square foot)					
1	226	79	4	17	99
2	89	67	1	32	95
3	28	57	14	29	60
4	26	46	12	42	58
5	72	76	7	17	38
6	215	71	18	11	27
7	494	83	9	8	10
8	346	53	38	9	10
9	325	51	30	19	9
DDT " Ditreen " (98 mg. DDT per square foot)					
1	185	22	2	76	40
2	81	20	1	79	19
3	24	12	17	71	22
4	53	51	8	41	10
5	185	56	21	23	14
6	375	58	16	26	20
7	1,076	55	11	34	13
8	708	43	24	33	18
9	1,043	36	17	47	19
DDT oil-bound suspension (309 mg. DDT per square foot)					
1	770	14	3	83	64
2	110	14	0	86	56
3	48	19	8	73	38
4	30	23	3	74	16
5	62	26	6	68	15
6	118	25	11	64	40
7	282	24	11	65	50
8	226	29	4	67	27
9	343	27	2	71	16
CONTROL					
1	407	45	42	13	8
2	197	49	44	7	6
3	98	29	62	9	10
4	127	36	61	3	14
5	175	67	29	4	4
6	293	71	27	2	8
7	1,032	67	31	2	4
8	421	52	45	3	11
9	798	26	70	4	5

*This includes mosquitos caught alive by hand in the hut in the morning and those found dead on the floor.

A. gambiae and *A. funestus* were maintained. More detailed examination of the figures showed that floor catches formed 72 per cent., 54 per cent., 36 per cent. and 8 per cent. of the total catches in months 1 to 4 respectively. Significantly higher kills than in the control hut occurred up to the sixth month. The highest proportion

of mosquitos in the morning window trap, 42 per cent., occurred in the fourth month, indicating that the insecticide was then only potent enough to irritate a large proportion of the mosquitos causing them to leave the hut without acquiring a lethal dose of the insecticide. Significant numbers of mosquitos began to appear in the evening window trap in eighth and ninth months, indicating that the effect of the insecticide had practically disappeared and that many of the mosquitos, which had entered the hut the previous night, had rested inside the hut throughout the following day without being irritated or killed.

TABLE IV.

Group I Experiments.

Results of applications of female *A. gambiae* and *A. funestus* under petri dishes to the walls of the treated and control huts.

Months after treatment	Application time in minutes	Percentage mortalities among female <i>A. gambiae</i> and <i>A. funestus</i> exposed to			Control
		BHC P.530	DDT "Ditreen"	DDT Oil-bound Suspension	
1	5	100	71	87	38
2	5	70	55	84	26
3	10	74	49	71	14
4	15	72	43	78	31
5	20	44	37	66	11
6	60	90	46	72	33
7	60	98	37	54	16
8	60	70	36	44	22
9	60	44	11	18	14

DDT Ditreen hut.

Kills among female *A. gambiae* and *A. funestus* were only 40 per cent. in the first month after treatment and considerably less in succeeding months, although usually higher than in the control hut, even up to the ninth month. Very few mosquitos were found dead on the floor of this hut (only 7 per cent. in the first month and less after this), and an average of 75 per cent. were found in the morning window trap during the first three months, suggesting a marked irritant property of this insecticide. A continued high percentage in the morning window trap, with only a small proportion occurring in the evening window trap, throughout the nine months of the observations, suggested that this irritant property was long lasting.

DDT oil-bound suspension hut.

Even at such a high dosage of DDT, about 40 per cent. of the female *A. gambiae* and *A. funestus* entering this hut escaped unharmed in the first two months after treatment and higher proportions after this. Significant kills are thought, however, to have been maintained up to the seventh month (the low kills in the third to fifth months were based on very small numbers). The percentage of mosquitos in the morning window trap remained very high throughout the nine months' observations, again suggesting the marked irritant properties of DDT. A very low percentage was found dead on the floor in the first four months (at the most 11 per cent. of the total mosquitos) and none after this. Very few mosquitos were found in the evening window trap throughout the experiments.

Further evidence of the irritant properties of DDT was afforded by the number of mosquitos caught flying in the morning inside the hut. Very few mosquitos were caught resting in either of the DDT huts over the whole period of the experiments,

whereas in the BHC hut, after the first two months, more and more mosquitos were caught resting at this time.

The results of the wall application tests (Table IV) revealed that the BHC formulation continued to produce nearly complete kills 7 months after treatment, when female *A. gambiae* and *A. funestus* were exposed for one hour, at a time when only a 10 per cent. kill was recorded among such mosquitos entering the hut normally. It is concluded, however, that this high kill produced in the narrow confines of the petri dishes in these later applications, was due for the most part to the fumigant effect of small quantities of the insecticide still remaining below the surface of the wall. In these tests higher kills were produced with both formulations of DDT than occurred in actual practice, and it is considered that normally the mosquitos are irritated and do not rest on treated surfaces for as long as one hour.

Nine months after the treatment of these huts, chemical analyses of portions of the walls still showed the presence of the insecticides in one-quarter inch scrapings, in the following quantities :—

BHC P. 530	... 13.7 and 21.0 mg. BHC (1.8 and 2.7 mg. of the gamma isomer) per sq. ft.
DDT "Ditrean"	... 62.6 mg. DDT per sq. ft.
DDT Oil-bound Suspension	... 75.4 mg. DDT per sq. ft.

It is almost certain, however, that these insecticides were not actually on the surface.

The results obtained in this first group of experiments do not bear out the findings of Muirhead Thomson (1950), who found almost no mortality among mosquitos entering and leaving a hut treated with DDT "Ditrean," even though the dosage was 400 mg. DDT per sq. ft. (this was not checked by chemical estimation, however). It is, however, clearly shown in the present experiments that DDT has more marked irritant properties than BHC and that quite a high proportion of the mosquitos entering huts treated with DDT escaped unharmed.

Laboratory studies by Hadaway and Barlow (1951a), have indicated that formulations of DDT containing large crystals are less efficient killers than those containing crystals of small size. The size of the particles of DDT "Ditrean" used in these experiments was found to be 60 μ while that of DDT oil-bound suspension was only 10 μ . This may account for the difference found in these experiments between the two DDT formulations, at least for three months after treatment, although the difference may have been entirely due to the marked difference in dosages. Evidence produced by the above two authors, however, shows that in the laboratory an increase in dosage of such small crystals above a small threshold does not increase the kill, although it may increase the persistence of the insecticide.

GROUP II EXPERIMENTS (Tables V and VI).

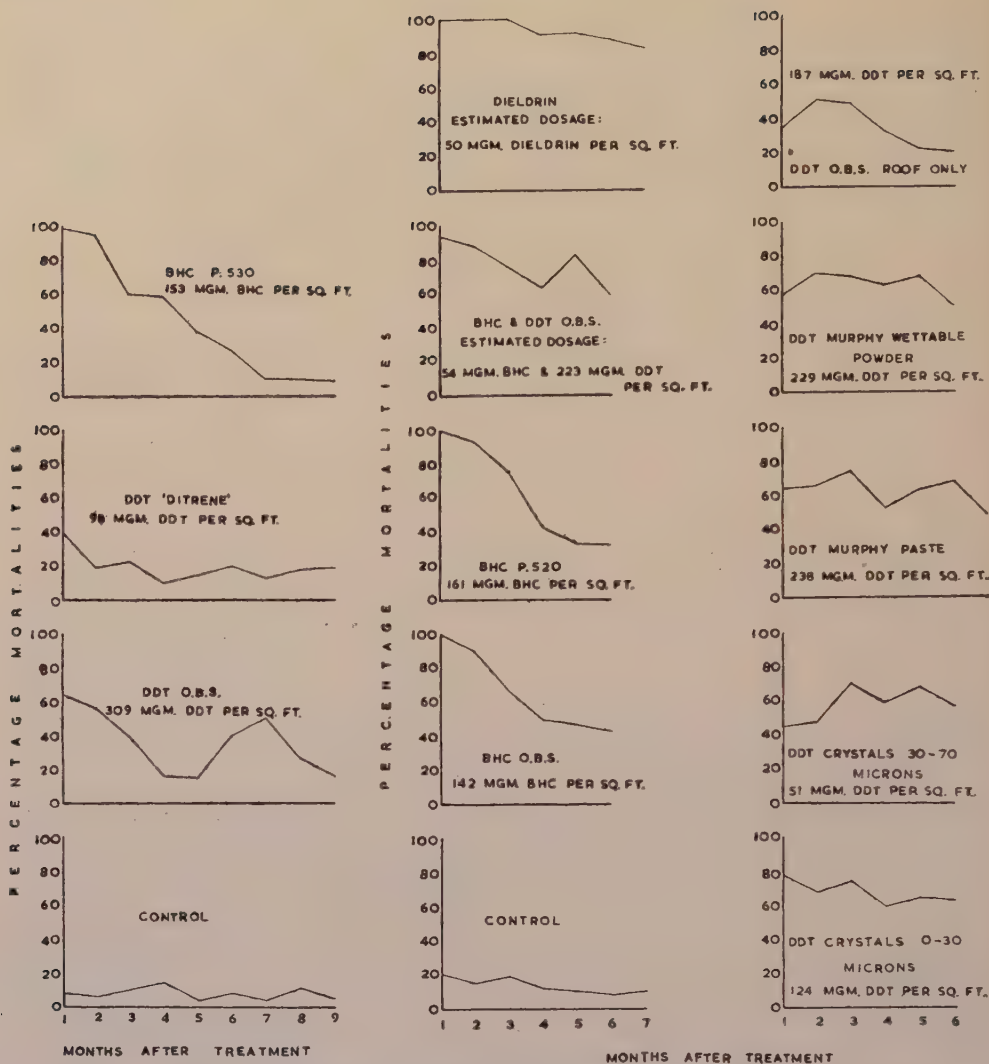
Three huts were sprayed in the middle of December, 1950, with the following insecticides :—

BHC oil-bound suspension "Supona" B (TP 725), containing 49.2 per cent. BHC, at an average dosage of 56.6 mg. BHC (7.4 mg. of the gamma isomer) per sq. ft.

BHC water-dispersible powder, P.520 (filler, diatomite), containing 47.9 per cent. BHC, at an average dosage of 34.2 mg. BHC (4.4 mg. of the gamma isomer) per sq. ft.

DDT Murphy paste (DDT content not chemically estimated—crystal size, 6 μ), at an average dosage of 74.5 mg. DDT per sq. ft.

In all three huts the insecticides gave significant kills for about one month only, indicating that these dosages are inadequate in practice. This short persistence



Graph 1.—Monthly percentage mortalities among female *A. gambiae* and *A. funestus* caught from treated and control huts. Group I experiments.

Graph 2.—Monthly percentage mortalities among female *A. gambiae* and *A. funestus* caught from treated and control huts. Group III experiments.

may also have been due to the soft absorbent wall surface employed in these huts. Observations were carried on for three months after treatment and, at the end of this time, all three formulations were still exerting some irritant effect as is shown by the high percentage of mosquitos in the morning window traps. This percentage was greater in the case of the DDT preparations, again indicating the more marked irritant properties of this insecticide.

A few wall application tests were carried out in these huts. In the BHC huts very low mortalities were obtained in the seventh week after treatment even with one hour's contact. Scraping the wall surface caused a significant increase in these mortalities, especially on the walls treated with BHC P.520, indicating that the

insecticide was still present below the surface. In the DDT-treated hut fairly high mortalities were maintained up to the ninth week and scraping of the wall surface again increased the mortality, but as indicated by the mortalities among mosquitos entering the hut naturally, the application times used in these wall tests are probably longer than would occur normally.

TABLE V.

Group II Experiments.

Monthly analysis of hut catches and mortalities among female *A. gambiae* and *A. funestus*.

Months after Treatment	Total Mosquitos	Percentage distribution of mosquitos			Overall percent- age mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		Inside hut	Evening window trap	Morning window trap	
BHC oil-bound suspension (57 mg. BHC per square foot)					
1	612	47	1	52	65
2	791	23	3	74	32
3	829	27	11	62	12
BHC P.520 (34 mg. BHC per square foot)					
1	724	42	3	55	84
2	858	32	5	63	25
3	1,146	33	16	51	15
DDT Murphy paste (75 mg. DDT per square foot)					
1	554	11	1	88	65
2	855	5	5	90	26
3	1,105	4	3	93	25
COMBINED CONTROLS					
1	1,503	37	53	10	18
2	1,335	26	64	10	8
3	607	40	48	12	3

TABLE VI.

Group II Experiments.

Results of applications of female *A. gambiae* and *A. funestus* under petri-dishes to the walls of the treated and control huts.

Weeks after treatment	Application time in minutes	Percentage mortalities among female <i>A. gambiae</i> and <i>A. funestus</i> exposed to			CONTROL
		BHC oil-bound suspension	BHC P.520	DDT Murphy paste	
6	10	17			6
7	60	13	33	70	15
*8	30	19	59	81	24
**9	60	3	13	52	4

*Walls scraped.

** Positions on walls changed.

GROUP III EXPERIMENTS.

In March, 1951, just at the start of the main mosquito season, nine huts were sprayed with the following insecticides :—

BHC water-dispersible powder, P.520, containing 47.9 per cent. BHC, at an average dosage of 161 mg. BHC (21 mg. of the gamma isomer) per sq. ft.

BHC oil-bound suspension "Supona" B containing 49.2 per cent. BHC, at an average dosage of 142 mg. BHC (18 mg. of the gamma isomer) per sq. ft.

A laboratory-made suspension of DDT crystals, <30 microns, at an average dosage of 124 mg. DDT per sq. ft.

A laboratory-made suspension of DDT crystals, 30–70 microns, at an average dosage of 51 mg. DDT per sq. ft.

DDT Murphy paste, containing 55.3 per cent. DDT, at an average dosage of 238 mg. DDT per sq. ft.

DDT Murphy wettable powder, containing 52.9 per cent. DDT, at an average dosage of 229 mg. DDT per sq. ft.

DDT oil-bound suspension "Supona" D containing 43.5 per cent. DDT, at an average dosage of 187 mg. DDT per sq. ft. This hut was sprayed on the roof only.

An oil-bound suspension "Supona" DB (TP 726) containing a mixture of BHC (13 per cent.) and DDT (40 per cent.) at an estimated dosage of 54 mg. BHC (7 mg. of the gamma isomer) and 223 mg. DDT per sq. ft.

The dosage of BHC and DDT from the mixture could not be estimated chemically and has been calculated from the following data :—

The average chemically-estimated deposit in the spraying of the DDT formulations, Murphy paste, Murphy wettable powder and the oil-bound suspension above was 212 mg. DDT per sq. ft., using 12 oz. of the formulations containing an average of 50.6 per cent. DDT, in one gallon of water.

Similarly the deposit in the spraying of the BHC P.520 and the BHC oil-bound suspension was 152 mg. BHC (20 mg. of the gamma isomer) per sq. ft., using 12 oz. of the formulations containing an average of 48.6 per cent. BHC, in one gallon of water. In spraying the mixture, 1 lb. was used in one gallon of water.

Dieldrin wettable powder containing 25.4 per cent. dieldrin at an estimated dosage of 50 mg. dieldrin per sq. ft. (Chemical analyses of sample papers from the dieldrin-treated hut, kindly carried out through Shell Co. Ltd., yielded the very low average figure of 7.2 mg. dieldrin per sq. ft. This is totally inconsistent with the estimated dosage.)

The results of these treatments are given in detail in the Tables and graphs and only a brief summary is given here.

BHC huts (Table VII, graph 2).

The results obtained in the two huts treated with BHC were very similar. Both showed 100 per cent. mortality among *A. gambiae* and *A. funestus* entering them for one month after treatment. Over 50 per cent. mortalities were maintained up to three months, and even in the fifth and sixth months mortalities remained between 30 and 40 per cent. as compared with control mortalities of about 10 per cent. (Table XI) in these months. During the period of complete kills, only about one-third of the mosquitos escaped as far as the window traps; most of them were found dead on the floor. As the mortalities decreased, higher proportions of the mosquitos reached the morning window trap, but after the fourth month these proportions decreased considerably, when mosquitos began to appear in significant

numbers in the evening window trap. Dead mosquitos continued to occur in significant numbers on the floors of these two huts even in the sixth month after their treatments.

TABLE VII.

Group III Experiments.

Monthly analysis of mosquito catches and mortalities in huts treated with BHC.

Months after treatment	Total mosquitos	Percentage distribution of mosquitos			Overall percentage mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		Inside hut	Evening window trap	Morning window trap	
	Hut treated with BHC P.520 (161 mg. BHC per square foot)				
1	340	56	0	44	100
2	1,060	37	0	63	93
3	929	33	1	66	76
4	350	24	17	59	43
5	392	24	52	24	33
6	425	19	55	26	32
	Hut treated with BHC oil-bound suspension, "Supona" B (142 mg. BHC per square foot)				
1	481	75	0	25	100
2	2,246	49	0	51	90
3	1,014	46	1	53	67
4	433	37	8	55	50
5	155	49	12	39	47
6	174	29	38	33	43

Results of the wall application tests (Table XIV) in these huts, indicated a marked decline in toxicity in the third and fourth months in the case of the oil-bound suspension, and in the fourth and fifth months in the case of the P.520, after one hour's application of the mosquitos.

DDT huts (Table VIII, graph 2).

All the huts which were treated completely with DDT showed general similarities and only minor differences. In all, at least 20 per cent. of the female *A. gambiae* and *A. funestus* escaped the effect of the insecticide and all showed its irritant properties, large proportions of the mosquitos appearing in the morning window trap. Very few mosquitos were found in the evening window trap up to five months after treatment. After this there was a slight increase in the evening window trap mosquitos, most marked in the hut treated with the DDT large-crystal suspension, indicating a decline in the toxicity of the formulations.

DDT small-crystal suspension.

This appeared to be the most efficient of the formulations. Mortalities were for the most part higher in this hut, as was the proportion of dead mosquitos found on the floor, while the proportion in the morning window trap was less.

DDT large-crystal suspension.

Mortalities were below 50 per cent. in the first two months after treatment but later increased to between 60 and 70 per cent. The proportion of dead mosquitos on the floor was correspondingly low at first but increased later. The percentage of mosquitos in the morning window trap was high for the first two months but

decreased in later months. The appearance of significant numbers of mosquitos in the evening window trap in the sixth month indicated a decline in the toxicity of the formulation.

TABLE VIII.
Group III Experiments.

Monthly analysis of mosquito catches and mortalities in huts treated with DDT.

Months after treatment	Total mosquitos	Percentage distribution of mosquitos			Overall percentage mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		Inside hut	Evening window trap	Morning window trap	
Hut treated with a DDT suspension of crystals, <30 microns (124 mg. DDT per square foot)					
1	353	61	3	36	78
2	551	53	2	45	68
3	641	50	0	50	74
4	946	45	1	54	60
5	286	50	1	49	64
6	251	48	9	43	63
Hut treated with a DDT suspension of crystals, 30-70 microns (51 mg. DDT per square foot)					
1	451	25	5	70	45
2	1,525	20	2	78	47
3	393	40	1	59	70
4	292	45	1	54	59
5	126	50	5	45	68
6	163	36	31	33	56
Hut treated with DDT Murphy paste (238 mg. DDT per square foot)					
1	462	25	5	70	64
2	2,571	16	1	83	66
3	3,821	21	1	78	74
4	2,003	24	1	75	53
5	527	42	3	55	63
6	279	39	7	54	68
7	533	28	9	63	48
Hut treated with DDT Murphy wettable powder (229 mg. DDT per square foot)					
1	1,001	23	1	76	57
2	4,369	19	1	80	70
3	1,359	29	1	70	68
4	726	40	1	59	63
5	219	45	4	51	68
6	342	34	16	50	51
Hut treated on the roof only with a DDT oil-bound suspension, "Supona" D (187 mg. DDT per square foot)					
1	1,649	22	1	77	35
2	5,356	10	0	90	51
3	1,644	11	1	88	48
4	416	19	14	67	33
5	150	14	40	46	22
6	169	17	53	30	20

Thus the results from these two huts confirm, in the first few weeks after treatment, that the smaller the crystal the higher the kill, as found by Hadaway and Barlow (1951a). After the first few weeks, the larger crystals possibly become smaller and these then produce a similar kill to that of the small-crystal suspension. The fact that the significantly smaller dosage of the large-crystal suspension continued to give as high kills as the small crystal one, would appear to support the findings of the above authors that, above a threshold concentration, mortality is independent of dosage.

DDT Murphy paste and Murphy wettable powder.

These two formulations produced almost identical results. Over 50 per cent. kills were maintained over a period of 6 months after treatment. Most of the mosquitos were found in the morning window trap, but there was a slight increase in the numbers of mosquitos found in the evening window trap in the sixth month in both cases, indicating a start in decline of toxicity of the formulations.

The results of applying mosquitos under petri dishes to the walls of the DDT-treated huts are given in Table XIV. The small-crystal suspension gave higher kills than the large-crystal one with one half and one hour's application up to four months after treatment, but about the same mortality occurred in the fifth and sixth months. The DDT Murphy paste and Murphy wettable powder gave nearly complete kills in the third to sixth months with one hour's application.

These kills were for the most part much higher than occurred normally in the DDT-treated huts. The time of contact under the petri dishes is considered to be much longer than would occur if the mosquitos had a chance to fly away (p. 250).

Hut sprayed with DDT oil-bound suspension on the roof only (Table VIII, graph 2).

Because most of the mosquitos resting in an untreated hut during the day-time are found on the roof, it was decided to see if the treatment of the roof alone would be as efficient a method of mosquito control as spraying the whole house. The wall surface was not protected to prevent spray from dropping on to it when the treatment was carried out.

Results show that this method only produced low kills, indicating that many of the mosquitos rest on the walls during the night. The kills of female *A. gambiae* and *A. funestus* varied between 30 and 50 per cent. in the first four months after treatment, but were seldom above 50 per cent. In the fifth and sixth months very low kills were obtained. In the first four months most of the mosquitos were found in the morning window trap and this proportion decreased in the fifth and sixth months, when significant numbers of mosquitos began to appear in the evening window trap. Floor catches averaged only 10 per cent. of the total catches during the first four months, while in the fifth and sixth months very few dead mosquitos were found.

Hut treated with a mixture of BHC and DDT (Table IX, graph 2).

Results show the initial very high kill of BHC (about 90 per cent. in the first two months) and the long-lasting moderately high kill of DDT (60 to 80 per cent. in the third to sixth months). The irritant properties of DDT are shown by the high proportion of mosquitos in the morning window trap throughout the period. Only small numbers of mosquitos were beginning to appear in the evening window trap in the sixth month and significant numbers of dead mosquitos continued to be found on the floor in this month. Almost complete kills were given in all the wall application tests (Table XIV).

Dieldrin hut (Table X, graph 2).

For three months after treatment complete kills of female *A. gambiae* and *A. funestus* were maintained in this hut, and between the third and seventh months

kills remained over 80 per cent. The proportion of mosquitos in the morning window trap was about 40 per cent. in the first month but rose to nearly 80 per cent. in the following two months, corresponding with the peak in the mosquito densities. Thereafter it fell to between 20 and 40 per cent. Large numbers of dead mosquitos were found on the floor of this hut throughout the seven months of the observations. Significant numbers of mosquitos began to appear in the evening window trap in the fifth month and increased in the following months, but the mortality among them remained high. Almost complete kills were given in all the wall application tests (Table XIV).

TABLE IX.

Group III Experiments.

Monthly analysis of mosquito catches and mortalities in hut treated with an oil-bound suspension of a mixture of BHC and DDT.

(Calculated dosage : 54 mg. BHC and 223 mg. DDT per square foot.)

Months after treatment	Total mosquitos	Percentage distribution of mosquitos			Overall percentage mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		Inside hut	Evening window trap	Morning window trap	
1	751	30	0	70	94
2	1,734	29	0	71	88
3	749	23	0	77	76
4	534	39	1	60	63
5	165	38	1	61	82
6	291	24	7	69	59

TABLE X.

Group III Experiments.

Monthly analysis of mosquito catches and mortalities in hut treated with dieldrin.

(Calculated dosage : 50 mg. dieldrin per square foot.)

Months after treatment	Total mosquitos	Percentage distribution of mosquitos			Overall percentage mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		Inside hut	Evening window trap	Morning window trap	
1	629	56	2	42	100
2	4,057	21	0	79	100
3	4,765	23	0	77	100
4	1,523	63	2	35	91
5	531	68	9	23	92
6	340	62	17	21	88
7	459	50	33	17	83

Comparison of the Effect of Insecticides on Female Anophelines (Table XII).

Little difference in the effects of the insecticides on *A. gambiae* and *A. funestus* females was observed in the third group of experiments. *A. gambiae* was slightly more susceptible to BHC in the fourth to sixth months after treatment than *A. funestus* while DDT was slightly more toxic to *A. funestus* than to *A. gambiae* throughout the period of the observations.

TABLE XI.

Group III Experiments.
Control hut. Monthly analysis of mosquito catches and mortalities.

Months	Total mosquitos	Percentage distribution of mosquitos			Overall percent- age mortality among female <i>A. gambiae</i> and <i>A. funestus</i>
		Inside hut	Evening window trap	Morning window trap	
1	1,487	50	40	10	6
2	6,682	33	14	53	15
3	4,993	16	16	68	19
4	1,610	24	37	39	12
5	1,170	31	63	6	10
6	602	31	68	1	8
7	852	37	59	4	10

TABLE XII.

Group III Experiments. Comparison of effect of the insecticides on *A. gambiae* and
A. funestus.

Monthly overall percentage mortalities among females caught from treated and control
huts.

Months after treat- ment	Percentage Mortalities							
	*BHC		**DDT		Dieldrin		Control	
	<i>gambiae</i>	<i>funestus</i>	<i>gambiae</i>	<i>funestus</i>	<i>gambiae</i>	<i>funestus</i>	<i>gambiae</i>	<i>funestus</i>
1	100	100	57	69	100	100	5	9
2	91	92	66	71	100	100	15	19
3	71	76	72	76	99	100	18	23
4	52	40	53	65	89	96	12	11
5	48	33	63	65	88	95	7	12
6	66	29	55	62	93	88	8	8
7			36	51	93	81	20	9

*Huts treated with BHC oil-bound suspension and P.520.

**Huts treated with DDT small- and large-crystal suspensions and with the two Murphy products.

Effect of Insecticides on Female Culicines.

In the third group of experiments almost all the Culicines entering the huts were *Culex fatigans*. Table XIII records the monthly mortalities among these Culicines. It can be seen that the DDT formulations, although they gave significantly higher kills than occurred in the control, seldom killed more than 20 to 30 per cent. of these mosquitos, even in the first week after treatment. BHC gave high kills for only one month and practically no kills after three months. Dieldrin, on the other hand, gave consistently high kills throughout the period of the observations.

The Fumigant and Particulate Effect of the Insecticides (Table XV).

High kills were obtained ten weeks after treatment, when female *A. gambiae* and *A. funestus* were suspended in cages inside the BHC-treated huts (BHC P.520 and BHC oil-bound suspension) for only one hour, without actual contact with the treated surfaces. Among those in cages near the wall, the nearest side of which was

TABLE XIII.

Group III Experiments.
Monthly analysis of percentage mortalities among female Culicines (mainly *C. fatigans*)
caught from treated and control huts.

Month	*BHC		**DDT		Dieldrin		Control	
	Total	% Mortality	Total	% Mortality	Total	% Mortality	Total	% Mortality
1	147	85	587	16	139	95	298	1
2	232	33	625	10	286	87	508	2
3	74	26	303	17	112	77	116	10
4	36	14	248	24	82	57	51	8
5	73	14	212	34	59	69	90	6
6	188	7	390	20	73	58	137	2
7					174	69	223	7

*Huts treated with BHC oil-bound suspension and P.520.

**Huts treated with DDT small- and large-crystal suspensions and with the two Murphy products.

TABLE XIV.

Group III Experiments.
Results of applications of female *A. gambiae* and *A. funestus* under petri dishes to the
walls of the treated and control huts—percentage mortalities.

Months after treatment ...	1	2	3	4	5	6
Application time in minutes ...	30	30	60	60	60	60
BHC oil-bound suspension ...	100	88	46	27	20	20
BHC P.520 ...	100	84	79	47	13	9
BHC & DDT oil-bound suspension ...	100	100	98	100	93	95
DDT small crystals ...	70	49	77	86	64	78
DDT large crystals ...	36	13	54	38	65	74
DDT Murphy paste ...	53	59	100	100	100	100
DDT Murphy wettable powder	98	98	100	100	97	100
Dieldrin ...	97	94	100	100	96	98
Control ...	15	10	3	8	16	13

quarter of an inch from the wall surface and the farthest side 3 ins. away, kills were almost complete. After three months, however, kills were low in both positions even when cages were left suspended overnight.

In the huts treated with DDT Murphy paste and Murphy wettable powder kills were for the most part low throughout the period of the experiments.

In the dieldrin-treated hut high kills among suspended mosquitos occurred throughout the six months' period of the observations, especially among those suspended near to the wall.

DDT and dieldrin are both relatively non-volatile insecticides, and kills produced among mosquitos suspended in huts treated with these insecticides without actual contact with the treated surfaces are probably due to the presence of airborne particles of the insecticides in the huts. If these particles occurred in similar numbers in both the DDT- and dieldrin-treated huts, higher kills would be expected in the dieldrin-treated hut because of the greater toxicity of this insecticide. Variation in kills might be explained by variations in microclimatic conditions inside the huts at the time of the experiments, e.g., variations in air currents dependent on external wind conditions.

Kills in the BHC-treated huts, where the insecticide is a volatile one, are probably due to a combination of fumigant and particulate effects.

TABLE XV.

Group III Experiments.

Percentage mortalities among female *A. gambiae* and *A. funestus* suspended in cages in treated and control huts without actual contact with the hut surfaces.

Weeks after treatment	Exposure time in hours	*BHC		*DDT		Dieldrin		Control	
		Near wall	Middle	Near wall	Middle	Near wall	Middle	Near wall	Middle
1	12	100	100	100	—	100	—	15	0
5	12	100	100	—	47	—	100	21	16
6	12	100	100	—	27	—	100	10	4
9	1	69	15	0	2	88	80	0	0
10	1	100	65	17	0	100	96	15	4
12	2	63	58	27	17	100	100	0	7
13	2	81	36	33	13	100	82	5	14
14	2	56	26	18	14	100	13	10	4
15	4	40	19	7	23	77	86	12	8
16	4	8	14	10	8	82	76	0	0
17	4	14	17	0	5	57	35	0	0
18	6	33	13	10	27	95	89	0	0
19	12	34	17	40	25	100	100	0	6
20	12	43	35	13	17	85	53	0	0
22	12	44	24	60	14	100	75	19	28

*The BHC formulations were P.520 and "Supona" B.

The DDT formulations were Murphy paste and Murphy wettable powder.

Dashes signify that no observations were made.

TABLE XVI.

Analysis of results of two-cage experiments over a period of two weeks after treatments.

Insecticide	Mosquitos tested	Percentage resting in treated cage	Average contact time in minutes	Percentage mortalities	
				Treated	Control
		<i>A. gambiae</i>			
BHC	104	48	22	75	41
DDT	114	59	8	32	19
BHC & DDT	11	64	8	57	33
Dieldrin	55	60	28	97	20
		<i>A. funestus</i>			
BHC	106	57	27	82	43
DDT	156	55	10	51	20
BHC & DDT	16	50	7	67	11
Dieldrin	38	50	36	94	22

Experiments to Determine the actual resting Time of Anophelines (Table XVI).

The apparatus used in these experiments consisted of two 1-ft.-cube chambers, lined with white cardboard, joined together by a perspex chamber of similar dimensions. Mosquitos blown gently from a sucking tube through a hole in the middle of the perspex chamber had the option of flying into one cardboard chamber or the other; the slippery nature of the perspex prevented them from settling for more than a few

moments in the perspex chamber. One of the pair of cardboard chambers had the whole of its internal surface (which was in panel form and removable) treated with insecticide while the other chamber was left untreated. It soon became obvious that there was no question of any of the insecticides used being repellent; at least as many mosquitos flew to the treated as to the untreated cage. Mosquitos which flew into the untreated cage invariably rested there for long periods, but were removed when they had rested there for as long as the average resting time of the mosquitos in the treated cage. Mosquitos entering the treated cage and resting there, were irritated after varying lengths of time and flew into the perspex chamber. This was taken as an escape reaction and, through a series of trap-doors, with the aid of sucking tubes, these irritated mosquitos were caught. The period of contact with the insecticide was noted. Both mosquitos from the treated cage and from the untreated one (as controls) were kept in small cages and the mortalities among them were recorded after 24 hours.

The dosages of the insecticides used in these experiments, as determined by chemical analyses, varied considerably from 28 to 86 mg. BHC (4 to 11 mg. of the gamma isomer) per sq. ft. in the BHC preparations, and from 67 to 207 mg. DDT per sq. ft. in the DDT preparations. The estimated dosage of dieldrin was 50 mg. per sq. ft. (again the chemically estimated dosage of 3.6 mg. was totally inconsistent). The results are a summary of three sets of experiments using the following formulations of insecticides; BHC oil-bound suspension, BHC P.530, BHC P.520, DDT oil-bound suspension, DDT Ditreen, DDT Murphy paste and wettable powder, BHC and DDT in oil-bound suspension and dieldrin wettable powder.

The results show conclusively that a high proportion of the mosquitos which had contact with DDT were irritated and flew towards light before they acquired a lethal dose. The contact with DDT was shorter than with BHC and dieldrin. The slightly longer contact of *A. funestus* with DDT probably accounted for the higher mortalities among this species.

The longest contacts were with dieldrin and mortalities were nearly 100 per cent. during the two weeks that observations were made. Again *A. funestus* was less easily irritated than *A. gambiae*.

Contact with BHC was slightly shorter than with dieldrin and a slightly higher proportion of the mosquitos escaped its killing action; *A. funestus* was again less easily irritated than *A. gambiae*.

In the few observations with the mixture of BHC and DDT, where, as will be recalled, DDT was the predominant insecticide, contact times before irritation and flying was induced were similar to those with DDT alone but, as would be expected, kills were higher due to the presence of BHC.

Mortalities in the controls were highest when BHC was used. This would be expected so short a time after treatment, taking into account the volatility of the insecticide and the close proximity of the untreated cage to the treated one. The comparatively low control mortalities when DDT and dieldrin were tested indicate that these insecticides have little or no fumigant action. The marked particulate effect of dieldrin would not be expected to occur as there would be little or no air movement in these small experimental cages.

The Fate of BHC and DDT applied to Mud Surfaces.

In the third group of experiments, small mud blocks, 3×3 ins., made of the same material as the walls of the huts and sprayed at the same time as the huts, were placed in the eaves and left. They were then chemically analysed about once a month after treatment to determine the proportion of the insecticides remaining

in the surface millimetre and that which had been absorbed into the remainder of the block. This could only be done for BHC and DDT.

As would be expected BHC showed a much more rapid loss from the surface than DDT, and that this was mainly a loss by volatilisation was indicated by the low rate of recovery from the surface and from the inside only three weeks after treatment. In the case of the DDT formulations such loss as there was appeared to be due mainly to absorption of the insecticide into the mud. In all DDT formulations, except the Murphy wettable powder, 50 per cent. of the insecticide remained on the surface after five months. In the case of the Murphy wettable powder, where the surface percentage remained over 70 per cent., the inert filler, absent in the other DDT formulations used, may possibly have held the insecticide on the surface. The small-crystal suspension of DDT did not disappear more rapidly from the surface than the large crystal one. This is not in agreement with the findings of Hadaway and Barlow (1951b). The material used in the present experiments, however, containing 50 per cent. gravel as it did, was probably less absorbent than the material used by these two authors.

Conclusions.

Dieldrin is undoubtedly the most efficient of the insecticides tested, although the dosage employed is not accurately known. It produced very high kills among female *A. gambiae* and *A. funestus* for longer than seven months. Its efficiency is due in part to its prolonged particulate effect.

Although the toxicity of dieldrin to man was no concern of the author in these investigations, it is thought wise to point out that, like DDT and BHC, dieldrin is toxic to mammals and proper precautions should be taken when applying this material to prevent contamination of the spray operators and other persons. Dieldrin has been widely used in the U.S.A. during the past two years for insect control purposes, and no harmful effects to users have been reported.

All the tests carried out with BHC at dosages over 100 mg. BHC (13 mg. of the gamma isomer) per sq. ft., indicate that significant kills of female *A. gambiae* and *A. funestus* occur only up to three or possibly four months after treatment. A similar persistence was recorded by this author against *A. moucheti* in the Belgian Congo (1950). BHC is the quickest acting of these residual insecticides immediately after application. Its fumigant and particulate action is marked but becomes insignificant after three or four months.

All the trials with DDT showed that, even immediately after its application, a significant proportion of the mosquitos escaped unharmed from huts treated with it. It has only a slight particulate effect. In general, small-crystal formulations of DDT give higher kills than large-crystal ones, at least shortly after treatment. Mortalities among female *A. gambiae* and *A. funestus* in huts treated completely with DDT usually remain over 50 per cent. for at least six months. At the end of this period a decline in toxicity is indicated by the appearance of significant numbers of mosquitos in the evening window trap.

A mixture of DDT and BHC has the advantage of giving a high kill initially, due to the BHC, and a long-lasting subsequent kill, due to the DDT.

Laboratory experiments by Hadaway and Barlow (1951b) on the sorption of solid insecticides by dried mud, showed that crystals of all the insecticides they used (various formulations of aldrin, BHC, dieldrin and DDT) rapidly disappear from the surface. Some of their tests were made with dried mud from Taveta with the same results. When crystals of DDT and dieldrin were no longer visible on the surface of the muds no kills were produced among *Aedes aegypti* exposed for long contact periods; but, when aldrin and BHC were used, kills continued

to be obtained though no crystals were visible, through the fumigant action of these two insecticides. Thus these authors conclude that "the difference in effectiveness of DDT and BHC wettable powders against *A. gambiae* in houses with mud walls in Africa may be accounted for partly, at least, by the rapid sorption of both insecticides into the wall resulting in the one case in a complete loss of toxicity and in the other a persistent fumigant effect." They go on to say that the "residual effects obtained with DDT wettable powders in houses of this type may be due only to the deposit on the roof."

The results obtained in the third group of experiments described in this paper do not support these findings. The chemical analyses of mud blocks show that something like 50 per cent. of the DDT formulations remain in the surface millimetre even after five months and fairly high kills among *A. gambiae* and *A. funestus* were maintained throughout the period in all the huts treated completely with DDT. Treatment of a roof only, as they suggested, gave lower mortalities than any other treatment used in these experiments.

The marked loss of BHC from the surface and its continuing killing action by the volatilisation of insecticide remaining below the surface, found by Hadaway and Barlow (1951b), is confirmed by the results of these experiments.

Summary.

Experimental huts similar in construction to the dwellings commonly used in East Africa, but with exit window traps, were sprayed with various formulations of the three residual insecticides, DDT, BHC, and dieldrin, and the effect on the *A. gambiae* and *A. funestus* entering them was observed.

The almost complete absence of kill recorded by Muirhead Thomson (1950) in experiments in similar huts in Tanganyika treated with DDT Ditreen was not confirmed by these experiments.

A significant proportion of the *A. gambiae* and *A. funestus* entering huts treated with DDT did, however, escape unharmed, even immediately after treatment, whereas with the other insecticides, BHC and dieldrin, none of these mosquitos escaped the effect at least in the first month after treatment.

In preliminary experiments in which observations were carried on for nine months after treatments, BHC P.530 still showed some effect after seven months. This was almost certainly due to the fumigant effect of the small amount of insecticide still remaining below the wall surface. The irritant properties of the two DDT formulations, Ditreen and the oil-bound suspension "Supona" D, still existed after nine months.

In a second group of experiments, dosages of less than 80 mg. DDT and less than 60 mg. BHC (8 mg. of the gamma isomer) per sq. ft. gave over 50 per cent. kills of *A. gambiae* and *A. funestus* for only one month.

In a third group of experiments, using two formulations of BHC, five of DDT, one of a mixture of DDT and BHC and one of dieldrin :—

- (a) Dieldrin was by far the most efficient insecticide and gave very high kills for over seven months.
- (b) The DDT formulations, Murphy paste, Murphy wettable powder, suspensions of DDT crystals $<30\ \mu$ and $30\text{--}70\ \mu$ in diameter, when applied to the whole internal surface of the huts, produced fairly high kills over the period of the observations (six to seven months), but significant proportions of the mosquitos escaped their action even immediately after treatment.
- (c) The BHC formulations, P.520 and the oil-bound suspension "Supona" B, gave high kills for three to four months only.

- (d) The mixture of BHC and DDT in oil-bound suspension "Supona" DB gave the high initial kill of BHC and the long-lasting moderately high kill of DDT.
- (e) Against *C. fatigans* all the DDT formulations used in the third group of experiments gave very low kills, the BHC formulations high initial kills and dieldrin high long-lasting kills.

BHC has marked fumigant and particulate properties lasting for three to four months. Dieldrin has a remarkable particulate action, which produces for the whole six-month period of the experiment, very high kills among mosquitos suspended without actual contact with the insecticidal surfaces ; DDT only shows this particulate effect to a slight extent.

It is probable that the differences in the toxicities to mosquitos of the insecticides used in these experiments is due partly to differences in the irritant properties of the insecticides. In the case of DDT many of the mosquitos having contact with this insecticide are irritated and escape from the treated surface before acquiring a lethal dose.

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1. Spraying the roof only of a hut with DDT.
2. Suspending cages of mosquitos inside huts to ascertain the fumigant and particulate effects of the insecticides.
3. Testing DDT preparations of known crystal size. The two DDT suspensions of small and large crystals used in the third group of experiments were kindly made for me in the laboratory at Porton.

I am also very grateful for advice and assistance in writing this article given by Mr. C. B. Symes, O.B.E., Officer-in-Charge Colonial Insecticides Committee, Colonial Office.

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References.

- DAVIDSON, G. (1950). A field study on "Gammexane" and malarial control in the Belgian Congo. II.—Ann. trop. Med. Parasit., **44**, pp. 1–26.
- HADAWAY, A. B. (1950). Observations on mosquito behaviour in native huts.—Bull. ent. Res., **41**, pp. 63–78.
- HADAWAY, A. B. & BARLOW, F. (1951a). Studies on aqueous suspensions of insecticides.—Bull. ent. Res., **41**, pp. 603–622.
- HADAWAY, A. B. & BARLOW, F. (1951b). Sorption of solid insecticides by dried mud.—Nature, **167**, p. 854.
- HADDOW, A. J. (1942). The mosquito fauna and climate of native huts at Kisumu, Kenya.—Bull. ent. Res., **33**, pp. 91–142.
- HOCKING, K. S. & MACINNES, D. G. (1948). Notes on the bionomics of *Anopheles gambiae* and *A. funestus* in East Africa.—Bull. ent. Res., **39**, pp. 453–465.

- THOMSON, R. C. MUIRHEAD. (1948). Studies on *Anopheles gambiae* and *A. melas* in and around Lagos.—Bull. ent. Res., **38**, pp. 527–558.
- THOMSON, R. C. MUIRHEAD-. (1950). DDT and Gammexane as residual insecticides against *Anopheles gambiae* in African houses.—Trans. R. Soc. trop. Med. Hyg., **43**, pp. 401–412.

POSTSCRIPT.

Observations have been continued in some of the huts of the third group of experiments since the writer left East Africa, and further results have been kindly forwarded by the Colonial Insecticide Research Unit, East Africa. They are very briefly appended here.

The huts treated with the DDT small- and large-crystal suspensions and with the two Murphy products continued to show mortalities among female *A. gambiae* and *A. funestus* entering them of between 40 and 60 per cent. up to nine months after their treatments, and only small numbers of mosquitos continued to be caught in the evening window trap. The Murphy wettable powder produced the highest mortalities in months six to nine and the Murphy paste the lowest. High proportions of mosquitos in the morning window trap continued in all these DDT-treated huts. Dead mosquitos continued to occur on the floors of all four huts in the ninth month after treatment.

Dieltzin continued to produce over 80 per cent. kills among female *A. gambiae* and *A. funestus* nine months after treatment. Most of the mosquitos continued to be found dead on the floor of this hut. The particulate effect of this insecticide was still very marked in the ninth month.



FIG. 1. The four huts used in group I experiments.



FIG. 2. Single hut with mud walls and a grass roof, showing the window trap in position.



FIG. 3. Hut used in group III experiments, with mud walls and a roof of palm thatch, showing the double doors and concrete canal.



FIG. 4. Another view of the hut used in group III experiments, showing the window trap in position.

STUDIES ON AQUEOUS SUSPENSIONS OF INSECTICIDES. PART IV. THE BEHAVIOUR OF MOSQUITOS IN CONTACT WITH INSECTICIDAL DEPOSITS.

By A. B. HADAWAY and F. BARLOW.

Colonial Insecticides Research Unit, Porton.

Kennedy (1947) first demonstrated in the laboratory that mosquitos are irritated by contact with DDT and may fly from the treated surface before acquiring a lethal dose. Muirhead Thomson (1950) showed that whereas practically all female *Anopheles gambiae* Giles escaped unharmed from an African hut treated with a DDT wettable powder, none escaped unharmed from a similar hut treated with a BHC wettable powder. Among the mosquitos that fed in the BHC-treated hut there was no indication of that irritation that drove them out of the DDT-treated hut. On the other hand, Wilkinson (1951) was unable to repeat these results in Uganda, and the differences in the kills of *A. gambiae* obtained with DDT and BHC were not so great as in Muirhead Thomson's studies. Results obtained by Wharton and Reid (1950) and Wharton (1951) with *A. maculatus* Theo. in Malaya agreed with those of Muirhead Thomson in showing a greater effectiveness of BHC but differed in that an appreciable number of mosquitos were killed inside the DDT-treated huts and that many of those escaping into the window traps had picked up a lethal dose.

Recent investigations have shown that the effectiveness of deposits from aqueous suspensions of DDT against mosquitos, *Aedes aegypti* (L.), is influenced by particle size and by the type of material to which they are applied (Hadaway & Barlow, 1951, 1952). Thus, the use of different formulations on various building materials may partly explain the conflicting field results. The experiments described in this paper were begun, therefore, in order to determine whether mosquitos would pick up a lethal dose of DDT from the most effective formulations before they were stimulated to fly from the treated surface, and to study the behaviour of mosquitos in contact with BHC and other compounds in use as residual insecticides. It was then thought that extension of these studies might possibly elucidate some aspects of the mode of action of insecticides, and a number of compounds not of practical importance were therefore included in the tests.

Results obtained by Wharton and Reid (1950) with *Culex fatigans* Wied. differed from those described for *Anopheles maculatus*. Female *C. fatigans* were irritated and driven out of the DDT huts and only a few died. Reid (1951) reported that further experiments "indicated a wide range of susceptibility to DDT as if there is almost a complete reaction spectrum with very susceptible species such as *A. maculatus* at one end and the least susceptible such as *C. fatigans* at the other". Reid also points out that "if a species of mosquito escapes unharmed from a hut treated with DDT this may be due to some aspect of behaviour which prevents, or allows only a very brief, contact with the treated surfaces, rather than to innate resistance". Experiments were also carried out, therefore, to determine whether there are any marked differences in the rate at which different species are irritated by contact with insecticidal deposits.

Materials and Methods.

The preparation of suspensions.

The compounds used were obtained in various ways. Pure pp' DDT, op' DDT, methoxychlor, gamma BHC, aldrin, dieldrin and compounds 711 and 269 were

obtained by crystallisation from the technical grades. DDD, DDE, $(p\text{-BrC}_6\text{H}_4)_2\text{CH.CCl}_3$, $(p\text{-ClC}_6\text{H}_4)_2\text{CH.CCl}_3$, $(\text{C}_6\text{H}_5)_2\text{CH.CCl}_3$ and $(p\text{-ClC}_6\text{H}_4)_2\text{CH.CH}_3$ were prepared by standard methods, while the rest of the compounds were kindly donated by various people.

Each compound was recrystallised before use; alpha- and beta-BHC from benzene, delta-BHC from chloroform-carbon tetrachloride and all the others from methanol or ethanol.

All the compounds were sprayed as suspensions of solid particles in 0.2 per cent. Teepol solution. A sufficient quantity of many of them was not available to allow grinding and separation by sedimentation to give fractions of the same particle size range for each compound. This would also have been very time-consuming and involved density measurements. Moreover, tests with various formulations of pp'DDT showed that crystal size and shape did not have any appreciable effect on the average time for activation, provided that the dosage of larger particles was sufficient to ensure that a mosquito made contact with some particles on landing. Suspensions of almost all the substances were made, therefore, by precipitation in water-ethanol-wetting agent mixtures.

The conditions for growth were controlled so that all the substances gave crystals of a size small enough to give an adequate coverage of the sprayed surface at 100 mg. per sq. ft. It is not intended to give details for every preparation as they varied greatly from one substance to another but the principles were as follows:—

The substance was dissolved at 2 per cent. concentration in ethanol or, if it was very insoluble in this medium, in acetone. This solution was added to a rapidly stirred solution of 1 per cent. Teepol in an ethanol-water mixture and stirring continued at a moderate rate for up to five hours or until crystallisation was complete. In general, the effect of increasing the ethanol-water ratio was to increase the growth rate and the crystal size. Therefore, in successive trials with each substance the proportion of ethanol to water was increased until the crystals obtained were still of a reasonably small size but growth was completed in a few hours.

This procedure is derived, of course, from that used by McIntosh (1947) for DDT but modified in the way just described to permit rapid examination of the crystal growth properties of the many substances tested. The suspensions obtained in this way were not very uniform in size because variables such as rate of stirring and temperature were not controlled but this was not important for our present purpose.

Having found the best conditions for crystal growth 100 mg. quantities of crystals were usually made for spraying. These were allowed to sediment, the supernatant liquid removed and the residue washed and resuspended three times in 0.2 per cent. Teepol solution. The volume was finally adjusted to 3.0 ml. and the whole suspension sprayed in the manner previously described (Hadaway & Barlow, 1951).

A few suspensions were not made in the manner just described. op'DDT would form only large crystals by growth in ethanol-water mixtures and so it was ground and a fraction containing less than 20 micron particles obtained by sedimentation. DFDT was dissolved in ethanol, and 0.2 per cent. Teepol solution added until the solution became milky in appearance, followed by slow crystallisation (*cf.* McIntosh, 1951).

Test insect.

The species used in these experiments was *Anopheles stephensi* List. A colony was established from eggs obtained from the Malaria Reference Laboratory, Horton Hospital, Epsom. Only female mosquitos, three days old, which had fed once on guineapigs were used in the tests.

Method of exposure to deposits.

The apparatus consisted simply of a number of perspex cylinders, 3 ins. long and 3 ins. in diameter. One end of each was open ; the other was closed with perspex except for a central aperture 0.5 in. in diameter. The test surface was clamped in a vertical position, and the open end of the cylinder was applied closely to it and prevented from rolling by small pieces of plasticine on the bench.

A general impression of the behaviour of mosquitos in contact with insecticidal deposits was obtained by the introduction of five mosquitos into the apparatus through the central aperture at the closed end. They preferred to rest on the vertical surface rather than on the curved, polished walls of the perspex tube, and after flying for a few seconds invariably alighted on the test surface. Even with only five mosquitos in the apparatus one would occasionally land close to another and disturb it so that it flew from the surface before coming to rest elsewhere. For this reason, any activity due to contact with deposits was exaggerated. The number of flights from the test surface during each minute, and the number of mosquitos at rest on the test surface at the end of each minute were recorded for a total of ten minutes. Carbon dioxide was then introduced into the apparatus, the mosquitos lightly anaesthetised and transferred to a recovery cage so that a mortality count could be made 24 hours later.

More detailed observations were made on individual mosquitos. A single mosquito was allowed to fly through the central aperture into the apparatus and after flying for a few seconds it invariably alighted on the test surface. On an untreated surface it would remain quiescent for long periods, usually for more than an hour. On some deposits the mosquito obviously became restless and sooner or later flew from the surface. As soon as this happened carbon dioxide was introduced into the apparatus and the mosquito transferred to a recovery cage so that death or survival after this single, timed contact could be recorded 24 hours later.

All tests were made in dim light at 78°F. and 65-70 per cent. relative humidity, and mosquitos were stored under the same conditions. When a series of compounds or formulations was tested on one day it was not possible usually to expose more than 10 mosquitos individually to each. Although numbers of mosquitos were therefore small, consistent results were obtained and these were confirmed when experiments were repeated.

The term activation is used in this paper. By this we mean stimulation to flight after only a short time in contact with a deposit and before toxic symptoms are evident.

DDT Formulations.*Commercial wettable powders on wallboard.*

A. stephensi females were stimulated to fly from wallboards after only a few minutes contact with deposits from aqueous suspensions of commercial wettable powders at a dosage of 100 mg. DDT per sq. ft. They repeatedly flew on and off the treated wallboards during a 10-minute exposure period but remained at rest on untreated ones. The average time to the first flight from a treated surface was approximately the same (4 minutes) for each formulation in all experiments, although the mean size of the insecticide particles varied considerably. In the experiment recorded in Table I the mean time to the first flight was the same for each formulation although there was more variation with B than with the other two. High kills occurred only after contact with deposits from formulation A which consisted of small particles with a mean size of 6 microns.

TABLE I.

Activity of *A. stephensi* on wallboards sprayed with aqueous suspensions of commercial wettable powders at a dosage of 100 mg. DDT per sq. ft.
(a) 5 mosquitos per test, sum of 3 replicates.

Formulation	Mean particle size in microns		Time in minutes										Percentage kill
			1	2	3	4	5	6	7	8	9	10	
A (75% wettable powder)	6	No. of departures from treated surface ...	1	0	1	8	16	14	15	14	13	14	87
		No. settled on treated surface ...	15	15	15	15	12	10	12	11	13	14	
B (50% wettable powder)	24	No. of departures ...	3	0	2	5	11	13	12	12	8	10	7
		No. settled ...	14	15	15	14	12	12	13	13	12	12	
C (50% wettable powder)	60	No. of departures ...	3	0	2	7	8	7	5	6	9	11	0
		No. settled ...	14	15	15	14	12	12	14	12	12	12	
Untreated	—	No. of departures ...	3	0	0	0	0	0	0	0	0	0	0
		No. settled ...	15	15	15	15	15	15	15	15	15	15	

(b) Individual mosquitos, 10 replicates.

Formulation	Mean time in minutes to first flight, and standard deviation	Percentage kill
A	4.2 ± 1.00	80
B	4.2 ± 2.12	10
C	4.3 ± 1.27	0

DDT crystals on dried mud.

Results similar to those given for commercial wettable powders on wallboards were obtained when *A. stephensi* made contact with Uganda mud blocks a few hours after they were sprayed with aqueous suspensions of DDT crystals at a dosage of 150 mg. per sq. ft. This dosage was used to ensure a more than adequate coverage by the 20–40 micron particles. Flight from the blocks treated with 20–40 micron particles occurred as soon as from those treated with 0–10 or 10–20 micron particles. Again, kills were obtained only after contact with the smallest particles (see Table II).

It is of interest to compare these results with those obtained at the same time with female *Aedes aegypti*, 3 days old and after one blood meal on guineapigs. Significant kills of the latter species occurred after contact with 10–20 micron particles as well as with 0–10 micron particles, although the average contact time was approximately the same for the two species. This may be accounted for by differences in behaviour. *Anopheles stephensi* remains stationary on an untreated surface, and although it soon becomes restless on DDT deposits and vigorously moves its legs, especially the hind pair, it usually does not change its position appreciably until it flies from the surface. On the other hand, *Aedes aegypti* usually walks about a great deal on the treated surface before flying off, and thus has greater opportunities of encountering more particles and picking them up more effectively.

TABLE II.

Exposures of individual mosquitos to mud blocks sprayed with aqueous suspensions of DDT crystals at a dosage of 150 mg. per sq. ft.

Species	DDT particle size in microns	Age of deposit	No. of replicates	Mean time in min. to first flight	Percentage kill
<i>Aedes aegypti</i>	0-10	Few hours	15	3.0 ± 1.12	93
	10-20		15	2.5 ± 0.80	73
	20-40		15	3.0 ± 1.39	0
<i>Anopheles stephensi</i>	0-10	Few hours	20	3.1 ± 0.96	65
	10-20		20	3.3 ± 1.15	5
	20-40		20	3.4 ± 1.01	0
<i>A. stephensi</i>	0-10	2 weeks	10	18, 21, 22, 24, 27, 28, 4 > 30	0
	10-20		10	4.9 ± 1.16	0
	20-40		10	4.6 ± 1.44	0
<i>A. stephensi</i>	0-10	4 weeks	6	> 30	0
	10-20		6	18, 22, 24, 3 > 30	0
	20-40		6	21 ± 4.5	0

The mud blocks used in these tests were stored at 78°F. and female *Anopheles stephensi* were exposed to them after 2 and 4 weeks. After 2 weeks only traces of the deposit could be seen microscopically on the surface of blocks treated with 0-10 micron particles. Some mosquitos remained in contact with these blocks for 30 minutes; others became restless and flew off after 18-28 minutes. Although an appreciable part of the deposit of 10-20 and 20-40 micron particles had disappeared, some were still visible, especially where there were clusters of particles formed by super-imposition of spray droplets. Mosquitos were irritated and stimulated to fly from these blocks treated with 10-20 and 20-40 micron particles after approximately 5 minutes.

After 4 weeks no deposit could be seen on the blocks treated with 0-10 micron particles, and only traces were visible microscopically on those treated with 10-20 and 20-40 micron particles. Mosquitos remained at rest on the surface of all blocks for long periods. No kills were obtained after contact with any of the blocks 2 and 4 weeks after treatment (see Table II).

This marked increase in the length of time mosquitos remained in contact with treated mud blocks after storage at 78°F. confirms previous observations that surface deposits of DDT are sorbed by dried mud and that 0-10 micron particles are sorbed much more rapidly than larger ones (Hadaway & Barlow, 1952).

DDT wettable powder on different materials.

An aqueous suspension of the 75 per cent. DDT wettable powder A, mean particle size of 6 microns, was sprayed on to mud blocks, wallboards, unpainted deal wood and glass plates. A few hours later contact with the deposits activated female *A. stephensi*, and flight from all four materials took place after approximately the same time. High kills were obtained, however, only after contact with deposits on mud blocks and wallboards (see Table III). Previous work has shown that deposits on these materials are readily available for pick-up by mosquitos and that deposits adhere more tenaciously to the surface of glass and deal wood.

It is concluded that *A. stephensi* females are irritated by contact with deposits from aqueous suspensions of DDT particles and fly from the treated surface after only a few minutes contact whether the particles are small (less than 10 microns)

TABLE III.

Exposures of individual *A. stephensi* to different materials sprayed with wettable powder A at a dosage of 100 mg. DDT per sq. ft.

10 replicates.

Material sprayed	Mean time in minutes to first flight	Percentage kill
Mud blocks ...	2.5 \pm 1.15	90
Wallboard ...	3.5 \pm 1.14	80
Unpainted wood ...	3.1 \pm 0.79	10
Glass ...	2.4 \pm 0.86	0

or larger (20–40 microns), and whether they are readily available for pick-up or stuck down. A lethal dose is acquired, however, only when the particles are in the 0–10 micron size range and are readily available. Negligible kills occur even after repeated landings on larger particles. Activation appears to be due, therefore, to penetration of the insecticide to “sense-organs” on the tarsi during the contact period while the lethal effects are due to the dosage of insecticidal particles picked up during the contact period and retained subsequently by the insect.

DDT, BHC and Dieldrin Wettable Powders.

Wallboards were sprayed with aqueous suspensions of a 75 per cent. DDT wettable powder (A), a 50 per cent. BHC wettable powder (P.520) and a 25 per cent. dieldrin wettable powder, and female *A. stephensi* were exposed to the treated surfaces a few hours later.

Mosquitos were irritated by contact with DDT deposits and flew from the surface after approximately the same time as in previous experiments. The average time to the first flight from the DDT-treated surface was 3.6 minutes, and a kill of 70 per cent. occurred (see Table IV). Mosquitos were also activated by contact with BHC deposits and flew from the surface earlier than they did from DDT deposits in an average time of 1.3 minutes. All of the mosquitos died very quickly after this brief contact with BHC deposits.

On the other hand, mosquitos were not stimulated to fly from the dieldrin deposits during a 30-minute contact period. All died during the 24 hours following this contact but death occurred very much later than it did after contact with BHC.

TABLE IV.

Exposures of individual *A. stephensi* to wallboards sprayed with aqueous suspensions of commercial wettable powders.

10 replicates.

Formulation	Dosage	Mean time in minutes to first flight	Percentage kill
75 per cent. DDT wettable powder	100 mg. DDT/sq. ft.	3.6 \pm 1.10	70
50 per cent. BHC wettable powder (P.520)	100 mg. BHC/sq. ft. (= 13 mg. γ -BHC)	1.3 \pm 0.37	100
25 per cent. dieldrin wettable powder	100 mg. dieldrin/sq. ft.	>30	100

DDT Analogues.

Aqueous suspensions of crystals of a number of DDT analogues were prepared by the methods described previously and sprayed on to wallboards at a dosage of 100 mg. per sq. ft. A few hours after application, *A. stephensi* females were allowed to alight individually on the deposits and the time to the first flight from each treated surface was recorded. Some physical properties of the compounds tested were measured and are given in Table V, together with data taken from the literature.

Substitution of other halogens for chlorine in the pp' positions on the phenyl groups of the DDT molecule produced marked changes in the rate at which mosquitos responded to contact with the deposits (Table VI). They were quickly irritated by contact with the fluorine analogue (DFDT) and flew off after a mean time of 0.7 minutes, compared with 4.5 minutes for pp' DDT. There was some indication of irritation on the bromine analogue, but all mosquitos remained on the iodine analogue for at least 30 minutes. Significant kills were obtained only after contact with pp' DDT and the bromine analogue. Solubility in petroleum ether and olive oil decreases, and the molecular size increases as this series is ascended from I to IV.

Mosquitos remained in contact with compound V (DDD), in which one of the three chlorine atoms attached to the ethane part of the DDT molecule is replaced by hydrogen, for a slightly longer time than with pp' DDT, but were stimulated to fly earlier, after approximately two minutes, from compound VI in which all three chlorine atoms attached to the ethane part of the molecule are replaced by hydrogen. Irritation was also quickly evident on deposits of compounds VII and VIII, in which the chlorine attached to one or both of the phenyl groups of the DDT molecule is replaced by hydrogen, and mosquitos flew from the treated surfaces after approximately two minutes. DDD was slightly less toxic than DDT, and compounds VI, VII, and VIII were non-toxic under the conditions of these tests. DDD is somewhat less soluble than pp' DDT, but compounds VI, VII, and VIII are all more soluble than pp' DDT, in petroleum ether or olive oil. It appears that the responses shown by mosquitos are not due to the presence of particular chlorine atoms on either the aliphatic or aromatic part of the DDT molecule.

In tests with a series of alkyloxy analogues (Compounds IX, X, XI and XII) the time to the first flight from the deposits increased as the molecular weight increased, and most mosquitos remained on the propoxy derivative for at least 30 minutes. The propoxy derivative is much more soluble than the other members of the series in petroleum ether. Both the methoxy and ethoxy analogues showed some toxicity to *A. stephensi* but no kills occurred after 30 minutes' contact with the propoxy derivative.

Mosquitos remained in contact with op' DDT (compound XIII) for approximately 7 minutes before flying from the treated surface. The change of one chlorine atom from the ortho to the para position in the DDT molecule resulted, therefore, in a delay in the responses shown by mosquitos and in a compound non-toxic to *A. stephensi* under the conditions of these tests. op' DDT is more soluble than pp' DDT in petroleum ether.

Mosquitos remained in contact for at least 30 minutes with compounds XIV and XV (DDE), both of which are more soluble than pp' DDT in petroleum ether and olive oil. Neither was toxic to *A. stephensi* under the conditions of these tests.

It may be concluded from the results with DDT analogues given in Table VI that a number of compounds whether toxic or not, are able to penetrate to "sense-organs" on the legs of mosquitos, cause irritation and stimulate the insects to fly from the treated surface. It is possible that lipoid solubility influences the rate at which this activation occurs in the series containing different halogens in the pp' positions, but for other compounds such as XII, XIV and XV solubility is not a limiting factor. In the di-alkyloxy series it may be the increasing size of the pp'

substituted groups which decreases the rate of access to the sense organs, while it is probable that there is something in the total molecular structure of XIV and XV which renders them inactive.

TABLE V.
DDT analogues and some physical properties.

No.	Compound	Common name	Melting point, °C. uncorrected	Solubility in petroleum ether, 60–80 gm./100 ml. 18°C.	Solubility. Literature figures
I	2,2-bis(p-fluorophenyl) -1,1,1 - trichloroethane	DFDT	42–43	100	>45.0*
II	2,2-bis(p-chlorophenyl) -1,1,1 trichloroethane	pp' DDT	109.5	4.0	10.5*
III	2,2-bis(p-bromophenyl) -1,1,1 - trichloroethane	—	141	1.2	2.0*
IV	2,2-bis(p-iodophenyl) -1,1,1 - trichloroethane	—	172.5–173	—	0.5*
V	2,2-bis(p-chlorophenyl)-1, 1 - dichloroethane	DDD	100	2.2	8.0*
VI	2,2-bis(p-chlorophenyl)-ethane	—	55–56	40.0	24†
VII	2-(phenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane	—	74–76	12.5	27.8*
VIII	2,2-bis-(phenyl) -1,1,1 -trichloroethane	—	53–53.5	27.0	33.7*
IX	2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane	Methoxychlor	88	2.2	9.7* 2†
X	2-(p-methoxyphenyl) -2- (p - ethoxyphenyl)-1,1,1-trichloroethane	—	84.5–85.5	2.1	
XI	2,2-bis(p-ethoxyphenyl) -1,1,1-trichloroethane	—	105–106	1.3	2†
XII	2,2 bis(p-propoxyphenyl)-1,1,1-trichloroethane	—	62	28.6	8†
XIII	2-(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane	op' DDT	73.5–74	12.2	
XIV	2,2-bis(p-chlorophenyl)-1,1,1,2-tetrachloroethane	—	93	15.0	
XV	2,2-bis(p-chlorophenyl) - 1,1 - dichloroethylene	DDE	88	10.0	21.1*

*von Oettingen and Sharpless (1946). gm./100 ml. olive oil at 37°C.

†Busvine (1946). gm./100 ml. P.31 oil.

BHC Formulations.

Contact and fumigant effects.

Wallboards were sprayed with aqueous suspensions of a 75 per cent. DDT wettable powder (A) and a 50 per cent. BHC wettable powder (P.520). When five mosquitos were exposed in the apparatus at the same time for a 10-minute period they repeatedly flew on and off the wallboards treated with DDT or BHC but remained at rest on untreated ones.

A piece of cotton mosquito netting (mesh 45) was stretched over the open end of each cylinder, and a perspex ring of the same diameter as the cylinder and 0.25 in. deep was inserted between the netting and the vertical wallboard. When mosquitos were then introduced into the apparatus, they alighted on the netting 0.25 in. from the deposit on the wallboard, and thus were prevented from making contact with the deposit yet were exposed to any vapour given off from it. Mosquitos remained at

TABLE VI.

Exposures of individual *A. stephensi* to DDT analogues applied at a dosage of 100 mg. per sq. ft. to wallboards.

10 replicates.

Compound No.	Particle size and shape, approximate	Mean time in minutes to first flight	Percentage kill
I	1-5 μ irregular particles	0.7 \pm 0.30	10
II	10-20 μ rods	4.5 \pm 0.87	70
III	2-20 μ rods	17, 20, 24, 7 > 30	60
IV	5-20 μ plates and rods	> 30	0
V	10-30 μ hexagonal plates	5.5 \pm 1.07	40
VI	30 μ aggregates, needles and plates	2.3 \pm 0.39	0
VII	20-30 μ needles	1.8 \pm 0.89	0
VIII	30-50 μ hexagonal plates... ..	2.0 \pm 0.66	0
IX	30 μ needles	5.4 \pm 1.39	40
X	10-30 μ plates	8.3 \pm 1.44	30
XI	10-20 μ plates	11.3 \pm 3.42	40
XII	30-50 μ aggregates of plates	26, 29, 8 > 30	0
XIII	< 20 μ ground particles	7.3 \pm 2.05	0
XIV	30-60 μ needles	> 30	0
XV	30 μ hexagons	> 30	0

rest on the netting for a 10-minute period and were not activated under these conditions when the wallboards were treated with DDT or BHC. There were no deaths when DDT-treated wallboards were used, but the fumigant properties of BHC are such that approximately 50 per cent. of the mosquitos died even though they had shown no symptoms of irritation during the exposure period.

Exposures of mosquitos for longer times to the vapour from wallboards treated a few hours previously with BHC wettable powder at 100 mg. BHC (=13 mg. gamma isomer) per sq. ft. showed that movement from the netting did not begin until after 24 minutes. Then, flight was not normal, toxic symptoms were evident, and mosquitos leaving the netting were knocked-down almost immediately. All mosquitos died after a 30-minute exposure period under these conditions. The results of these tests are given in Table VII.

It is concluded that contact with fresh deposits of BHC quickly irritates female *A. stephensi* and stimulates them to fly from the treated surface, and that the vapour from BHC deposits has no irritant effect until the mosquitos are already showing toxic symptoms.

BHC isomers.

Aqueous suspensions of pure isomers of hexachlorocyclohexane were sprayed on to wallboards at a dosage of 100 mg. per sq. ft. Female *A. stephensi* were soon activated by contact with the delta isomer and flew from the treated surface after an average time of 1.3 minutes: all survived. The average time to the first flight from the gamma isomer was 4.7 minutes, and all mosquitos died very soon after the single contact. On the other hand mosquitos were not stimulated to fly from deposits of either the alpha or beta isomer during a 30-minute contact period nor were these isomers toxic to *A. stephensi* under the conditions of the tests. Some physical properties of the isomers are also given in Table VIII.

TABLE VII.

Exposures of *A. stephensi* to wallboards sprayed at 100 mg. DDT or BHC per sq. ft.
(a) Five mosquitos per test ; sum of three replicates.

Treatment	Type of exposure		Time in minutes										Per-centage kill
			1	2	3	4	5	6	7	8	9	10	
Untreated	Contact	No. of departures	3	0	0	0	0	0	0	0	0	0	0
		No. settled ...	15	15	15	15	15	15	15	15	15	15	
75 per cent. wettable powder (A)	Contact	No. of departures	2	1	7	11	8	10	23	14	20	20	87
		No. settled ...	15	15	14	11	14	11	9	10	13	10	
50 per cent. BHC wettable powder (P.520)	Contact	No. of departures	4	11	19	21	24	23	17	25	20	17	100
		No. settled ...	12	14	14	13	12	13	12	13	13	13	
A	Fumigation*	No. of departures	2	0	0	0	0	0	0	0	0	0	0
		No. settled ...	15	15	15	15	15	15	15	15	15	15	
P.520	Fumigation*	No. of departures	4	0	0	0	0	0	0	0	0	0	53
		No. settled ...	15	15	15	15	15	15	15	15	15	15	

(b) Five mosquitos per test ; sum of four replicates.

Treat-ment	Type of exposure		Time in minutes												Per-centage kill
			1	2	3	4-22	23	24	25	26	27	28	29	30	
P.520	Fumigation*	No. of departures...	3	0	0	0	0	4	3	1	1	2	1	3	100
		No. settled ...	19	20	20	20	20	19	18	18	17	16	16	16	
		No. knocked-down ...	0	0	0	0	0	0	1	2	3	4	4	4	

*Cotton netting 0.25 in. from deposit, preventing contact.

TABLE VIII.

Exposures of individual *A. stephensi* to wallboards sprayed with aqueous suspensions of BHC isomers at a dosage of 100 mg. per sq. ft.

10 replicates.

Isomer	Particle size	Melting point, °C. uncorrected	Solubility. Literature figures	Mean time in min. to first flight	Percentage kill
Alpha	1-10 μ plates and rods	156-156.5	1.5*	>30	0
Beta ...	1-2 μ chunky crystals	309	0.7*	>30	0
Gamma	10-50 μ hexagons ...	112.5	5.2*	4.7 \pm 1.02	100
Delta ...	1-2 μ chunky crystals	137-137.5	13.4*	1.3 \pm 0.73	0

*Gm./100 gm. petroleum oil, grade 5 (Armstrong & others, 1951).

P.520 on mud.

A Uganda mud block was sprayed with a water suspension of P.520 at a dosage of 100 mg. BHC per sq. ft. Six female mosquitos allowed to alight individually on the

block 1 to 2 hours after treatment flew from the surface in the following times :—

0.8, 2.5, 14.7, 13.7, 16.6 and 18.8 minutes.

Other mud blocks were sprayed with the same formulation at a dosage of 200 mg. BHC per sq. ft. and stored at 78°F. Five female *A. stephensi* were allowed to alight individually on the treated surface at different time intervals after the application. One hour after treatment all were soon irritated and flew from the surface after an average time of 1.6 minutes. Four to five hours after treatment mosquitos remained in contact with the surface for varying times. One day and one week after treatment all mosquitos remained in contact with the treated surface for 30 minutes or more, and when they did fly off were obviously showing toxic symptoms and were soon "knocked-down".

It has been shown previously (Hadaway & Barlow, 1952) that surface deposits of BHC are rapidly sorbed by mud and soon disappear, and that the sorbed insecticide continues to exert a fumigant effect. This phenomenon therefore, provides an explanation for the results given in Table IX. Initially, mosquitos are activated by contact with deposits, and the presence of the delta isomer can account for the rapidity with which activation occurs. After sorption has taken place, contact is prevented and mosquitos are not disturbed in the presence of BHC vapour until they show toxic symptoms. It is likely that the insecticide is not distributed evenly over the whole surface of the mud and that the sorption process is completed in some areas before it is in others. At this stage, mosquitos may, by chance, either make contact with the insecticide and be activated after only a few minutes or be subjected only to the vapour from the sorbed insecticide. This would explain the variable results obtained at a dosage of 100 mg. per sq. ft. and four to five hours after treatment at a dosage of 200 mg. per sq. ft.

TABLE IX.

Exposures of individual *A. stephensi* to mud blocks sprayed with P.520 at a dosage of 200 mg. BHC (= 26 mg. gamma isomer) per sq. ft.

Five replicates.

Age of deposit	Mean time in minutes to first flight	Percentage kill
1 hour	1.6 ± 0.76	100
4-5 hours	3.3, 3.1, 4.5, 15.7, 18.5	100
24 hours	29 ± 2.6	100
1 week	36 ± 2.4	100

P.520 on other materials.

Wallboards, Whatman No. 1 filter papers, squares of unpainted deal wood and glass plates were sprayed with an aqueous suspension of BHC wettable powder P.520 at a dosage of 100 mg. BHC (= 13 mg. gamma isomer) per sq. ft., and stored at 78°F. Female *A. stephensi* were allowed to alight individually on the surfaces at various time intervals after treatment.

A few hours after treatment mosquitos were soon irritated and flew from all surfaces after average times of from 1 to 2 minutes. This rapid activation can be accounted for by the presence of the delta isomer. The time to the first flight from a treated surface increased as the deposits aged and varied with the different materials (see Table X).

There is evidence that loss of BHC from the surface of wallboards and filter papers occurs by sorption as well as by evaporation, although the sorption process is at a slower rate than on mud. The time to the first flight from wallboards and filter

papers varied two days after treatment and then increased considerably as the deposits aged. When mosquitos eventually flew from these surfaces they were usually already showing toxic symptoms and were soon knocked down. This is indicative of fumigation.

Results obtained with glass plates, where there is no sorption, can be accounted for by evaporation of the insecticide from the surface and the consequent reduction in the dosage available to give activation and kills. Deposits on unpainted deal wood behaved more like those on glass than on wallboards and filter paper.

TABLE X.

Exposures of individual *A. stephensi* to different materials sprayed with P.520 at a dosage of 100 mg. BHC per sq. ft. and stored at 78°F.

Material	Age of deposit	No. of replicates	Mean time in minutes to first flight	Percentage kill
Wallboard	Few hours	8	1.5 ± 0.39	100
	1 day	8	2.9 ± 0.90	100
	2 days	6	2.3, 4.5, 4.7, 5.8, 13.5, 14.3	83
	4 days	6	15 ± 2.9	100
	7 days	6	22 ± 1.5	83
	15 days	6	42 ± 2.5	83
Filter paper	Few hours	8	1.7 ± 0.64	100
	1 day	8	2.5 ± 0.94	100
	2 days	6	2.2, 4.5, 4.7, 10.7, 13.3, 14.2	83
	4 days	6	6.8, 8.0, 10.5, 12.3, 15.2, 17.5	83
	7 days	6	12, 29, 4 > 30	83
	15 days	6	6 > 45	50
Unpainted wood	Few hours	8	1.1 ± 0.41	100
	1 day	8	1.5 ± 0.73	100
	2 days	6	5.0 ± 2.40	83
	4 days	6	5.3 ± 2.06	83
	7 days	6	13, 14, 21, 3 > 30	50
	15 days	6	41, 5 > 45	0
Glass	Few hours	8	1.2 ± 0.45	100
	1 day	8	1.1 ± 0.42	100
	2 days	6	5.0 ± 1.77	100
	4 days	6	4.8 ± 2.02	17
	7 days	6	7.1 ± 1.99	17
	15 days	6	14.5 ± 4.17	0

Chlordane, Related Compounds and Toxaphene.

Tests in which five mosquitos were exposed in the apparatus at the same time indicated that female *A. stephensi* are not activated by contact with deposits of α -chlordane, dieldrin, aldrin, compounds 269 and 711 (stereoisomers of dieldrin and aldrin respectively) and toxaphene (see Table XI).

This was confirmed when individual mosquitos were allowed to alight on deposits from aqueous suspensions of the pure compounds and of commercial wettable powders. Mosquitos remained in contact with the deposits of pure compounds for at least half an hour. When they did eventually fly from wallboards treated with aldrin and chlordane wettable powders they were obviously showing toxic symptoms. This may be accounted for by the fumigant properties of these insecticides and that a lethal concentration of vapour accumulated in the apparatus during the relatively long exposure period. Commercial dieldrin wettable powder contains impurities with

TABLE XI.

Activity of *A. stephensi* on wallboards sprayed with aqueous suspensions of insecticides at a dosage of 100 mg. per sq. ft.

(a) Five mosquitos per test : sum of three replicates.

Insecticide		Time in minutes										Percentage kill
		1	2	3	4	5	6	7	8	9	10	
α -chlordane	No. of departures	3	0	0	0	0	0	0	0	0	0	67
	No. settled ...	15	15	15	15	15	15	15	15	15	15	
aldrin	No. of departures	2	0	0	0	0	0	0	0	0	0	100
	No. settled ...	15	15	15	15	15	15	15	15	15	15	
dieldrin	No. of departures	1	0	0	0	0	0	0	0	0	0	100
	No. settled ...	15	15	15	15	15	15	15	15	15	15	
compound 269	No. of departures	4	0	0	0	0	0	0	0	0	0	100
	No. settled ...	15	15	15	15	15	15	15	15	15	15	
compound 711	No. of departures	2	0	0	0	0	0	0	0	0	0	100
	No. settled ...	15	15	15	15	15	15	15	15	15	15	
toxaphene	No. of departures	3	0	0	0	0	0	0	0	0	0	0
	No. settled ...	14	15	15	15	15	15	15	15	15	15	

(b) Individual exposures : six replicates.

Insecticide	Formulation, particle size	Melting point, °C. uncorrected	Solubility in pet. ether 60-80 gm./100 ml., 18°C.	Mean time in min. to first flight	Percentage kill
α -chlordane	10 μ plates and rods	104-105	11.1	>60	100
aldrin ...	10-20 μ plates ...	101-102	84.2	34	100
dieldrin ...	20-30 μ needles ...	176	4.0	43	100
compound 269	5 μ rods ...	decomp.	5.3	55	100
compound 711	1-5 μ rods ...	decomp.	15.8	48	100
chlordan	50 per cent. wettable powder	—	—	25	100
aldrin ...	20 per cent. wettable powder	—	—	33	100
dieldrin ...	25 per cent. wettable powder	—	—	33	100
toxaphene	25 per cent. wettable powder	—	—	>30	0

fumigant properties and the build-up of a concentration of vapour may explain the difference in the times to the first flight from pure dieldrin and from the commercial wettable powder. Technical chlordane in the commercial wettable powder is a mixture of various isomers including α -chlordane. The difference in the time to the first flight from deposits of chlordane wettable powder and α -chlordane indicates that the latter is one of the lesser biologically active constituents of technical chlordane.

Aldrin, dieldrin and chlordane are highly toxic to mosquitos and complete kills were obtained after all the individual exposures. No kills occurred after 30 minutes' contact with toxaphene. Some physical properties of the compounds used are given in Table XI. It is noted that aldrin is highly soluble in petroleum ether.

Different Species of Mosquitos.

Anopheles stephensi List.

Unfed females, 2 days old, were activated by contact with deposits from the 75 per cent. DDT wettable powder A as rapidly as were fed females, 3 days old. The mean time to the first flight from the treated surface was 2.9 ± 1.03 mins. for unfed females and 3.7 ± 0.85 mins. for fed females. Ninety per cent. of the unfed mosquitos died, compared with 70 per cent. of the fed ones.

A. gambiae Giles.

Adults were reared from eggs sent by air from Nigeria. Three-day-old females after one blood meal on a human arm were used in the tests.

A. quadrimaculatus Say.

Adults were reared from eggs obtained from the colony maintained at the Malaria Reference Laboratory, Horton Hospital, Epsom. Three-day-old females after one blood meal on guineapigs were used in the tests.

Culex pipiens molestus Forsk. (London strain).

Adults were reared from eggs obtained from the colony maintained at the Malaria Reference Laboratory, Horton Hospital, Epsom. Unfed females, 2-3 days old, were used in the tests.

A. maculipennis var. *atroparvus* van Thiel was found to be unsuitable for tests of this nature for under these conditions it usually flew even from an untreated surface after less than a minute contact.

Results given in Table XII show that there were no marked differences in the rates at which these species were activated by contact with a given insecticide. All mosquitos died after contact with fresh deposits of BHC and dieldrin, but no *C. p. molestus* died after a single contact with DDT deposits even though these consisted of particles of less than 10 microns.

TABLE XII.

Exposure of individual mosquitos to wallboards sprayed with wettable powders.

Formulation	Species	No. of replicates	Mean time in minutes to first flight	Percentage kill
DDT wettable powder A	<i>A. stephensi</i> ...	10	3.6 ± 1.10	70
	<i>A. gambiae</i> ...	15	2.4 ± 0.76	53
	<i>A. quadrimaculatus</i> ...	10	3.3 ± 1.02	60
	<i>C. p. molestus</i> ...	15	1.8 ± 0.51	0
BHC wettable powder P.520	<i>A. stephensi</i> ...	10	1.3 ± 0.37	100
	<i>A. gambiae</i> ...	15	0.7 ± 0.26	100
	<i>A. quadrimaculatus</i> ...	10	0.5 ± 0.21	100
	<i>C. p. molestus</i> ...	15	0.9 ± 0.32	100
Dieldrin wettable powder	<i>A. stephensi</i> ...	10	>30	100
	<i>A. gambiae</i> ...	10	26 ± 2.6	100
	<i>A. quadrimaculatus</i> ...	10	25 ± 3.4	100
	<i>C. p. molestus</i> ...	10	>30	100

Discussion.

Laboratory experiments have demonstrated that *Aedes aegypti* (L.) and *Anopheles maculipennis* var. *atroparvus* van Thiel (Kennedy, 1947) and *A. quadrimaculatus* Say (Fay & Sheppard, 1949) are activated by contact with DDT. There is ample evidence from field work to show that many other species of Anopheline and Culicine mosquitos are irritated by contact with DDT and that they may be driven from treated dwellings before acquiring a lethal dose, so that the effectiveness of DDT residual sprays is greatly impaired. Experiments described in this paper show that, although activation of *A. stephensi* List. occurs with all DDT formulations tested on various materials, a mosquito can acquire a lethal dose before it is stimulated to fly from the treated surface provided that the insecticide particles are small enough and are readily available for pick-up. Kills were negligible after contact with 0-10 micron particles adhering to the surface and after repeated landings on available larger particles.

Baranyovits (1951) found that, whereas house-flies remain comparatively quiescent on deposits of gamma BHC and do not leave the surface until they have acquired a lethal dose, they are obviously disturbed on deposits of DDT and leave the surface much earlier and mainly survive. Preliminary tests with *Aedes aegypti* showed similar results on a much reduced time scale. Various workers have found no evidence of irritation of mosquitos in BHC-treated houses, the majority of mosquitos being found dead on the floor and only a few reaching the window traps (Muirhead Thomson, 1950 ; Bertram, 1950 ; Wharton, 1951).

The results of our laboratory experiments with BHC are more difficult to describe than are those with DDT because evaporation and fumigant effects have to be taken into consideration. Interpretation of results is also complicated by the presence of the delta isomer, which causes activation, as well as the gamma isomer which is responsible for toxicity. It is evident, however, that *Anopheles stephensi* is irritated by contact with fresh deposits of BHC wettable powder and is stimulated to fly from the treated surface after only 1 to 2 minutes. Gamma-BHC has such a high toxicity to mosquitos, however, that even when contact is for a brief period only they die very quickly. On the other hand, mosquitos are not disturbed by the vapour from BHC deposits, when contact is prevented, until they are obviously showing toxic symptoms and just before they are knocked down. The time mosquitos remain in contact with BHC-treated surfaces increases as the deposit ages and varies according to the type of material sprayed. Thus, on dried mud blocks, sorption of surface deposits takes place rapidly and mosquitos subjected to the fumigant action of the sorbed insecticide usually remain in contact with the mud surface until just before they are knocked down. Entry of mosquitos into the window traps of experimental African huts treated with BHC wettable powder would not, therefore, be expected.

The following description by Downs & Bordas (1951) of the behaviour of *A. pseudo-punctipennis* Theo. in houses with adobe walls and thatched roof treated with BHC wettable powder is suggestive of fumigation : " Mosquitos show much less irritation during the course of gammexane intoxication (than with DDT), and when they do begin to fly about, usually apparently at an advanced stage of intoxication, they do not necessarily head towards light ".

Particle size of DDT was more critical for *A. stephensi* than for *Aedes aegypti*. This may be accounted for partly by differences in behaviour while in contact with deposits, for a mosquito which walks about a great deal has greater opportunities of encountering more particles and picking them up more effectively than one which stands still.

Other tests showed that there were no very marked differences in the rate at which a few species of mosquitos were activated by DDT or BHC. The numbers used were too small to draw any conclusions about differences in susceptibility to DDT of the Anopheline species, but it appears significant that no kills of *C. p. molestus* occurred

after contact with deposits from a wettable powder consisting of particles less than 10 microns. Several authors have found that residual spraying of houses with DDT has been less effective against some Culicine species than against Anophelines. Reid (1951) has found that mosquitos in Malaya show a wide range of susceptibility to DDT and that *C. fatigans* is the least susceptible species.

Results of early experiments suggested that the flight response shown by mosquitos in contact with DDT or BHC followed penetration of the insecticide to "sense organs" on the tarsi. It was thought, therefore, that the time to the first flight from a compound would be a useful measure of its rate of penetration and that studies with a number of closely related compounds might provide information on some of the factors governing the first stage in the penetration of insecticides through the insect cuticle.

It was found that *A. stephensi* was activated by contact with a number of compounds whether they were toxic or not. In the series of DDT analogues containing different halogens in the pp' positions, and in the series of BHC isomers, lipid solubility appeared to be an important factor in determining the rate at which activation occurred. Armstrong, Bradbury and Standen (1951) studied the penetration of BHC isomers through the cuticle of grain weevils and concluded that the first stage of pick-up of insecticide by the insects is simple solution of the insecticide in the outer waxy covering of the epicuticle. They found that the amount of each isomer taken up by the insects was in the approximate ratio of their solubilities in hydrocarbon solvents.

Lipoid solubility is not, however, the limiting factor in other series. In the di-alkyloxy series, for example, the increasing size of the pp' substituted groups retards penetration although the propoxy derivative is much more soluble than the methoxy and ethoxy analogues.

Results obtained with other DDT analogues also suggested that total molecular structure plays an important part in determining the rate of access of a compound to the sense organs and whether it will stimulate a mosquito to flight. Mosquitos flew from deposits of pp' DDT sooner than they did from deposits of the more soluble op' isomer. Krijgsman and Lingbeek (1951) compared the contact toxicity and real toxicity, as determined by injection, of p-, m- and o-iodonitrobenzene and their dichlorides. Although the real toxicity of the p- compounds was only slightly higher than that of their isomers, their contact action was very much higher than that of their isomers. It was concluded that the position of the iodine atom in relation to the nitro group affects the permeation velocity very considerably. In this example, too, the p-iodonitrobenzene is generally less soluble than the o-isomer. Evidently molecular shape is more important than solubility as with pp' and op' DDT.

Summary.

The behaviour of mosquitos in contact with deposits from aqueous suspensions of insecticides has been studied by allowing them to alight individually and measuring the time to the first flight.

Anopheles stephensi females flew from all formulations of DDT on various materials after approximately the same time of 2 to 4 minutes, but subsequent kills were high only when the deposit consisted of small (less than 10 microns) particles readily available for pick-up.

Mosquitos were also activated by contact with fresh deposits of BHC wettable powder P.520, but all died very quickly. They were not disturbed, however, by the vapour from BHC deposits, when contact was prevented, until in an advanced stage of intoxication. The time to the first flight from surfaces treated with BHC wettable

powder increased as the deposits aged and varied with different materials according to the rate of loss by evaporation and/or sorption.

Mosquitos remained in contact with deposits of chlordane, dieldrin, aldrin and toxaphene for more than 30 minutes.

Experiments with a number of DDT analogues and BHC isomers indicated that lipid solubility and molecular structure are two factors governing the rate of penetration of a compound.

The reactions of other species tested were similar to those of *A. stephensi* but kills of *Culex p. molestus* were significantly different from those of the Anopheline species after contact with deposits from a DDT wettable powder.

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References.

- ARMSTRONG, G., BRADBURY, F. R. & STANDEN, H. (1951). *Ann. appl. Biol.*, **38**, pp. 555-566.
- BARANYOVITS, F. (1951). *Nature*, **168**, pp. 960-961.
- BARLOW, F. & HADAWAY, A. B. (1952). *Bull. ent. Res.*, **42**, pp. 769-777.
- BERTRAM, D. M. (1950). *Ann. trop. Med. Parasit.*, **44**, pp. 242-254.
- BUSVINE, J. R. (1946). *J. Soc. chem. Ind.*, **65**, pp. 356-360.
- DOWNES, W. G. & BORDAS, E. (1951). *Amer. J. Hyg.*, **54**, pp. 150-156.
- FAY, R. W. & SHEPPARD, E. H. (1949). *J. nat. Malar. Soc.*, **8**, pp. 147-158.
- HADAWAY, A. B. & BARLOW, F. (1951). *Bull. ent. Res.*, **41**, pp. 603-622.
- HADAWAY, A. B. & BARLOW, F. (1952). *Bull. ent. Res.*, **43**, pp. 281-311.
- KENNEDY, J. S. (1947). *Bull. ent. Res.*, **37**, pp. 593-607.
- KRIJGSMAN, B. J. & LINGBEEK, T. (1951). *Bull. ent. Res.*, **42**, pp. 135-141.
- MCINTOSH, A. H. (1947). *Ann. appl. Biol.*, **34**, pp. 586-610.
- MCINTOSH, A. H. (1951). *Ann. appl. Biol.*, **38**, pp. 881-898.
- VON OETTINGEN, W. F. & SHARPLESS, N. E. (1946). *J. Pharmacol.*, **88**, pp. 400-413.
- REID, J. A. (1951). *Nature*, **168**, pp. 863-865.
- THOMSON, R. C. & MUIRHEAD. (1950). *Trans. R. Soc. trop. Med. Hyg.*, **43**, pp. 401-412.
- WHARTON, R. H. (1951). *Bull. ent. Res.*, **42**, pp. 1-20.
- WHARTON, R. H. & REID, J. A. (1950). *Nature*, **165**, pp. 28-29.
- WILKINSON, P. R. (1951). *Bull. ent. Res.*, **42**, pp. 45-54.
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A CRITIQUE ON THE TIME FACTOR IN BIOLOGICAL CONTROL.

By Wendell F. SELLERS, B.Sc.

Commonwealth Institute of Biological Control.

During the year 1951 two articles appeared on The Time Factor in Biological Control (Clausen, February ; Thompson, September). The first was the 1950 Presidential Address by C. P. Clausen to the annual meeting of the American Association of Economic Entomologists. The second was a commentary on and criticism of Clausen's theses by W. R. Thompson. The main point made by Clausen on the basis of available published field observations was that any introduced entomophagous parasite or predator that is to be effective will achieve full commercial control within three host generations, or at the most within three years, in the vicinity of the colonisation points. Thompson examined Clausen's arguments in relation to the general theory of biological control and endeavoured to demonstrate their plausibility by restating the problem in mathematical form. He made reservations in regard to certain points but stated that Clausen's views constitute a working hypothesis that merits careful examination and may have an important influence on biological control work in the future.

Both papers contain many interesting and important points. The purpose of the present paper is to discuss them in the light of experience gained by the writer during some 29 years' work in the field.

It has frequently been pointed out that insect behaviour is often inexplicable because the insects have not had an opportunity to become familiar with the articles, bulletins and books that have been written about them. This remark emphasises the lack of the sound ecological observations on which biological control work should be based. This lack occurs either because the observations are not made, or because some of the directing agencies insist on spreading the work of their personnel over an area that is too large to permit adequate investigation of all the complexities involved.

Perhaps the basic principle in all work on populations of living organisms is the one adopted by Darwin (1859) from Malthus : that each organism is striving to increase in geometrical ratio, and that if it fails by itself to cover the habitable globe this is because during some season of the year in each generation, or at intervals, it suffers great destruction. Biological control work depends on this principle in that it endeavours to cause and control the destruction of noxious organisms by the use of their natural enemies, *i.e.*, any organism which habitually injures or destroys them.

Entomologists have long been impressed by the rapidity with which, under certain circumstances, insects multiply to pest status. A corollary of this is the rapidity with which, under favourable conditions, certain natural enemies, including parasites and predators, bring the pests under control. As Thompson has pointed out, we are almost always forced to ascribe the limitations of the numbers of pests to a combination of factors, biotic and physical, varying from time to time and from point to point. In an important paper Solomon (1949) has stressed and supported this view which primarily emphasises the influence of environment correlated with time. It implies that the major factors contributing to the control of a pest in its native home are not necessarily biotic. Nevertheless, we have abundant evidence that biotic factors are frequently very important. These factors are often specific in their action so that a species that is accidentally or purposely transported to a distant area may escape from them. The objective of biological control work in

general is to re-establish the natural balance by bringing the biotic factors once more into operation.

Thompson subjects Clausen's thesis to a mathematical test, using formulae derived from those published in his 1922 and 1923 papers in which the numerical relations equivalent to control are represented by the formulae :

$$n = ps^t \text{ and } t = \frac{\log n/p}{\log s}$$

In these formulae, n represents the number of individuals in the host population, p the number of individuals in the parasite population, s the effective reproductive rate per generation and t the number of generations. After making some computations with the help of these formulae, Thompson finds himself essentially in agreement with Clausen. "This", he says, "is a case in which mathematical theory and reality coincide for all practical purposes. These curious and rather surprising consequences follow of course from the simple fact that we are dealing with geometrical progression or, in other words, with the growth of something whose rate of increase is proportional to its dimensions at that moment." To put the matter in another way, Clausen's axiom, since it is dealing with quantitative values, is a mathematical axiom, and Thompson has shown how it can be justified on mathematical grounds.

The term "full commercial control" used by Clausen is defined by him as meaning the degree of control which renders other measures unnecessary. This means, of course, that the economic necessities of the situation are satisfied by the degree of control achieved. We must agree that whether we attain it or not, this is the real objective of our biological control work. There is, however, not necessarily any relationship between the mere prevention of increase produced by natural control and conditions that are commercially satisfactory. In Holland, the biological control of the pine shoot moth, *Rhyacionia buoliana* (Schiff.), on Scots pine is seldom achieved before the woodland is rendered commercially and aesthetically useless. Since the Scots pine has not in fact been annihilated, we must admit that the pest has been controlled by a combination of biotic and physical factors. The fact that the natural control in this case is of no commercial value appears to be related to the poor quality of the soil of the heathlands used for the pine plantations. In Central Europe and in England, on better soils, control that is satisfactory commercially and aesthetically is ordinarily obtained. The evidence indicates that the more suitable the soil for the growth of Scots pine, the better the results achieved by the controlling agencies among which the biotic factors play an important part. The parasitic enemies of the pine shoot moth are abundant in Holland, but an inspection of local conditions might lead one to conclude that they are of no value anywhere. Investigation in Central Europe and England shows that this idea is incorrect, but it also makes clear the fact that the value of a parasite or predator cannot be regarded as a constant, but varies according to the importance and composition of the general background of controlling factors.

Clausen has endeavoured to give exact definitions of effective, partially effective, and ineffective natural enemies. This classification restricts the applicability of his general conclusions. Certain difficulties arise from the fact that many of the parasites and predators cited as examples could under different environmental conditions be placed in any of the three classes mentioned. Clausen is careful to point out that in any particular case the degree of control obtained depends on conditions. He confines his discussion almost exclusively to cases of effective control by a single parasite or predator. Thompson has suggested that reference might have been made to "the idea that by the accumulation of a number of species attacking different stages or attacking the same stage in different micro-environments, a controlling complex might eventually be built up", but in support of Clausen, he cites the outstanding biological control of the pest weed, *Cordia macrostachya* (Jacq.), in Mauritius by the

introduction from the West Indies of the Galerucid beetle, *Schematiza cordiae* Barber. Another example of the biological control of a weed pest is that of the prickly pear by *Cactoblastis cactorum* (Berg) in Australia, and yet another is the control of the weed *Hypericum* in Australia, California and Oregon by *Chrysomela quadrigemina* Suffr. and *C. hyperici* Forst. All these cases concern control by one or two species and usually by one alone.

Although the specific characteristics of insect parasites, like those of other organisms, are somewhat variable and some species that are distributed over a large and diversified area seem to have local races whose peculiarities can be correlated with local conditions, Clausen and Thompson agree that there is no evidence of progressive adaptation capable of transforming an ineffective parasite into an effective parasite. Thompson suggests, however, that in some cases more time may be required to obtain decisive results than Clausen allows. Clausen's conclusions are based on the proviso that colonisation and establishment shall be adequate. What actually constitutes adequate colonisation and establishment is a very controversial question. Until we know exactly what these terms mean in a practical operation we cannot feel entirely satisfied that Clausen's theses are valid. L. O. Howard and others have suggested that the parasite may need time to become adapted to a new environment. Perhaps the real point that they are making is that time may be required in certain instances to obtain adequate establishment in the form of an equilibrium of distribution, after which the parasite can respond effectively.

In order to bring out the complexity of the problem we are discussing, it seems necessary to comment on certain of the examples cited by Clausen. The control of the cottony cushion scale, *Icerya purchasi* Mask. by the ladybird, *Rodolia cardinalis* (Muls.) is a classic in the history of biological control. There is no doubt that this Coccinellid quickly saved the California citrus industry from the destruction that threatened it. However, it now appears that the control of the scale by *Rodolia* is not so complete and consistent as the textbooks usually suggest. It seems that at the present time the parasitic Agromyzid, *Cryptochaetum iceryae* (Will.), is just as important a controlling agent of the cottony cushion scale as *Rodolia*.^{*} *Rodolia* is more effective in the inland areas, whereas *Cryptochaetum* is more valuable in the coastal districts. Generally speaking, the heavier outbreaks of the cottony cushion scale in California are on ornamentals, but in recent years, owing to the use of powerful insecticides and the inadvertent elimination of its natural enemies, severe outbreaks have occurred in some orchards, necessitating additional commercial treatments. In some of the orchards not subjected to the additional treatments, the natural enemies re-established themselves after the residual effect of insecticides disappeared, and this establishment was followed by rapid control. In any event, the observations made on the cottony cushion scale and its natural enemies during recent years indicate that the biological control of this species, though reasonably satisfactory, is somewhat variable.

That the citrus mealybug, *Pseudococcus citri* (Risso) is not permanently kept in commercial control in California by the ladybird, *Cryptolaemus montrouzieri* Muls. is indicated by the fact that the mass production of the ladybird is still going on. This may be regarded as a precaution against a sudden outbreak due to conditions exceptionally favourable to the mealybug. It may also be taken as evidence that the normal field population is not always adequate. It certainly seems to indicate a doubt about the ability of *Cryptolaemus* to maintain permanent commercial control of the citrus

^{*}In Bermuda, infestation by the cottony cushion scale increases during the winter months on ornamentals, especially the shrub *Pittosporum*. This is because *Rodolia* does not work effectively during the Bermuda winter. In 1950-51, the Commonwealth Institute of Biological Control made 16 large shipments of field-collected cottony cushion scale to Bermuda. This material was heavily parasitised by *Cryptochaetum*, of which over 18,000 adults were colonised in all the parishes of Bermuda. The reports received indicate that *Cryptochaetum* has become established, but its controlling value has not yet been determined.

mealybug. The course of events indicated that *Cryptolaemus* was more useful against *Pseudococcus gahani* Green until superseded by internal parasites introduced as a means of control.

In California, the parasite *Metaphycus helvolus* (Comp.) is certainly an efficient control of the black scale, *Saissetia oleae* (Bern.), in some seasons. However, the cold winters of 1948-49 and 1949-50 disrupted the normally synchronised relationship between these two insects so that the black scale returned to pest status. In this case, the host and parasite responded in different ways to the cold winter so that the effectiveness of the parasite was reduced. No doubt with the return of milder winter the control of *Saissetia* by *Metaphycus* will improve.

Observations made during a visit to Mexico in 1946 suggested to the writer that the parasite, *Eretmocerus serius* Silv., introduced from Cuba, was not controlling the citrus black fly, *Aleurocanthus woglumi* Ashby, as had been anticipated. Under Cuban conditions, it was effective but in Mexico it was ineffective. The Coccinellid predator, *Catana clauseni* Chapin, was, according to Clausen, highly effective in reducing heavy initial infestation of the black fly in Cuba, but was of little value in maintaining it at low level thereafter. The ladybird, *Cryptognatha nodiceps* Mshl., introduced from Trinidad into Fiji for the control of the coconut scale, *Aspidiotus destructor* Sign., was a great success in Fiji, but as Thompson has pointed out, it is not outstanding among the natural enemies of the pest in the West Indies.

There are three noteworthy features that are common to most of the cases of successful biological control cited by Clausen and by Thompson, and above by the writer.

1. In addition to a satisfactory environment and host relationship, an initially high host population favoured the natural enemy by allowing it more nearly to approach its potential reproductive rate. With effective biological control and a great reduction in host numbers, we may expect the reproductive rate and effectiveness of the natural enemy to decline somewhat. The host may remain under commercial control but at a somewhat higher level of population than before. In cases such as those cited above, the more violent the outbreak, the more impressive is the initial control effected. The interrelations of certain endemic or native pests and parasites follow the same pattern. A marked outbreak is brought rapidly under control, and the pest population then oscillates at a relatively low level. In the case of insect pests, predators seem to be more affected by the fall in host density than parasites. Similar results are often obtained in the biological control of weeds by phytophagous insects. The following list gives instances where the relationships correspond to the pattern just described.

PEST	EFFECTIVE INSECT ENEMY
<i>Icerya purchasi</i> Mask. (Cottony cushion scale).	<i>Rodolia cardinalis</i> (Muls.). <i>Cryptochaetum iceryae</i> (Will.).
<i>Pseudococcus citri</i> (Risso) (Citrus mealybug).	<i>Cryptolaemus montrouzieri</i> Muls.
<i>Saissetia oleae</i> (Bern.) (Black scale).	<i>Metaphycus helvolus</i> (Comp.).
<i>Aleurocanthus woglumi</i> Ashby (Citrus black fly).	<i>Catana clauseni</i> Chapin.
<i>Pseudococcus kenyae</i> Le P. (Coffee mealybug).	<i>Anagyrus kivuensis</i> Comp.
<i>Pieris rapae</i> (L.) (Cabbage worm). Prickly pear.	<i>Pteromalus puparum</i> (L.). <i>Cactoblastis cactorum</i> (Berg).
<i>Hypericum</i> (Australia).	<i>Chrysomela quadrigemina</i> Suffr. <i>Chrysomela hyperici</i> Forst.

Additions can probably be made to this list when more careful field observations have been carried out. The minor decline in the intensity of action of the natural enemy need not surprise us. It has often been noted that when insects or plants are introduced into new habitats and become pests there, the intensity of the initial outbreak is seldom maintained. Introduced natural enemies sometimes follow the same course, though in this case it appears to be a result of the fall in host density which they themselves have produced. As this factor cannot be cited in the case of the hosts, the problem may be more complex than has been realised.

2. It is noteworthy that all the successful cases of biological control cited above involve only a single species of parasite or predator. If two species are involved, one is usually dominant. The facts collected by Clausen certainly show that in some cases one or at most two introduced natural enemies can produce effective control, but the existence of these cases is no proof that, in general, the introduction of sequences of parasites and predators is unnecessary.

3. When natural enemies are colonised, the time required for effective biological control appears to depend essentially on the rate of dispersal of the introduced parasite or predator. A slow rate of dispersal of the colonised insect enemy may result in successful control in three generations or less, especially if there are several host generations per annum and the effective reproductive rate of the parasite exceeds that of the host. Under these conditions, the host supply tends to a maximum while the increase of the parasite occurs in a restricted area where it is immediately effective. A rapid rate of dispersal extends the time necessary for successful control. In this case, the geometrical-ratio increase of the natural enemy takes place over a wide area. It is not until an equilibrium of distribution is established that the maximum effects of this increase are obtained in the original colonisation area. No cases of effective control of a host which has only one generation a year by an introduced enemy which also has only one generation a year were cited by Clausen.* It is unlikely that a parasite with only one generation per annum and having also a rapid rate of dispersal could achieve full commercial control in three years, and it is well to remember that environmental resistance is greater to insects with long generations than to those with short generations, and that the chance of survival of insects with a fast rate of dispersal is lower than that of those with a slow rate.

It is not suggested that any of the remarks so far made invalidate Clausen's three-generation, three-year thesis as based on the factual data he gives. They do, however, limit or qualify to some extent our ideas on the degree of effectiveness of the control in respect to oscillating host populations as well as on the ability of the natural enemies to compensate for an increase in environmental resistance. As has already been stated, the value of a parasite or predator should be regarded, not as a constant, but rather as a variable influenced by the importance and composition of the other interacting factors. More often than not, the natural enemies respond quite differently from the host to the influence of the same stimuli, and often when the response is the same, the results conflict.

To be regarded as effective, natural enemies should produce control annually or within the season. This is the basic premise on which all mass-production programmes designed to increase annually the effectiveness of indigenous or established parasites must stand or fall. As the potential reproductive rate of insects is

*The Dipterous parasite, *Centeter cinerea* Aldr., and its host, the Japanese beetle, *Popillia japonica* Newm., each having only one generation a year, constitute an example of this type of relationship. Clausen (1951) discusses the general disappointment that, after its introduction into the United States, this seemingly effective Japanese parasite has only qualified as an ineffective parasite. Two European Dipterous parasites, *Sturmia scutellata* (R.-D.) and *Parasetigena silvestris* (R.-D.), introduced into the United States against the gipsy moth, *Porthetria dispar* (L.), are additional examples that will be discussed in this paper. These two parasites are equally effective in both habitats.

very high, it is easy to obtain from small populations high densities in the following generation or from a few individuals high densities in the third generation. The time involved in this effective control is obviously shorter than the three host generation, three-year period. It is equally obvious that Clausen's minimum periods are only obtained when "effective" parasites or predators are involved. When the natural enemies are initially being established we are confronted with a time lag. The two principal factors involved in this time lag are the initial distribution and the rate of dispersal. These both affect the building up of a controlling density of the parasite or predator.

W. F. Fiske described dispersal following colonisation as comparable to the pattern caused by dropping a stone into the still waters of a pond, the successive ripples representing the spread in the successive generations.

Thompson offers a theoretical treatment to explain the local concentration of parasites within the area of parasite distribution following liberation. He simplifies the treatment by postulating that the host population is uniformly distributed and that the parasite population spreads backward and forward from a point on a base line, migrating in each generation a definite distance perpendicular to this line. He points out that there is a concentration near the base line for a considerable period. "Nevertheless", he states, "on the postulates adopted above, the distribution of the parasites is much less uniform than might be anticipated with respect to the host population, even within the limits of their dispersion". If the flight lines discussed in this treatment are realigned as concentric rings around a central point, similar to the pattern which distribution from a colony centre may reasonably be expected to follow (and similar to the distribution pattern suggested by Fiske),

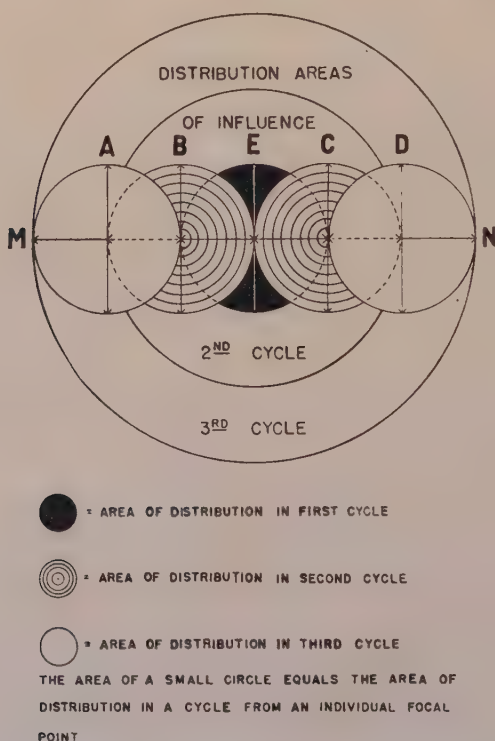


Fig. 1.—Theoretical Distribution Diagram of the initial three generation cycles following colonisation of a natural enemy.

much the same lack of uniformity in the distribution pattern will be obtained, but the parasite population of the concentric flight lines will be equal to the total population of the plus and minus flight lines.

The present writer has had under consideration for many years a treatment of distribution that helps to explain the time lag in the effective introduction of natural enemies. The formulation is a refinement of Fiske's and Thompson's treatments to demonstrate an equilibrium of distribution. The broad postulate is that an "equilibrium of distribution" must be established at the colony site (first parasite generation distribution area) before the pest is exposed to the full effectiveness of the reproductive increase of the introduced insect. This equilibrium is obtained when there is no fall in the numbers of the parasite due to outward dispersal into the uncolonised area; the backward and forward surges of dispersal offset each other, producing by themselves a minimum uniformity or stability of distribution.

Let us assume that the host population is uniformly distributed and that the parasite population within a constant flight radius also distributes itself uniformly (fig. 1). The initial point or centre is the colony site, and the general direction of the distribution is outward during the first generation. In any subsequent generation, outward distribution or dispersal means in any direction into the uncolonised area. Inward dispersal means movement in any direction inside the already colonised area. From any point on the periphery of the area of distribution, more than one-half of the dispersal is outward. From points within the area, there is some overlapping of the inward and outward surges which are neutralising each other and producing stability in the sense that there is no loss of parasites by outward dispersal. With each subsequent generation, the limit of the area of parasite dispersal outward will move farther and farther from the colony site, but there will occur simultaneously parasite dispersal inward that will establish an equilibrium of distribution in the zone of influence of the preceding generation. It is obvious that with the passage of successive generations, the dispersal or movement from an ever increasing number of points will be entirely expended within the central area, but this effect is over and above that required for an "equilibrium of distribution".

Fig. 1 is a simple diagram showing the theoretical distribution of a natural enemy after its colonisation in a new habitat in relation to its initial three generation cycles on a uniform host population.

The point of colonisation is where ordinate E bisects the abscissa MN. In the first generation or cycle, the parasite population attains an area of distribution represented by the blacked-in circle but with a relatively low density. In the first generation, the general direction of the dispersal of the parasite population is outward from the point of colonisation.

In the second generation, the overall area of distribution increases to four times that of the first-generation area. From an indefinite number of points in the first generation area, the outward dispersal is into the uncolonised area and the inward dispersal is within the first-generation area of distribution. As the area of the second-generation distribution is three times that of the first-generation, the effect of the increase of the outwardly dispersing parasite is more or less nullified by the additional area colonised. In the first-generation area, where the inward and outward surges of dispersal meet or are neutralised, an "equilibrium of distribution" is produced. Using only three selected focal points where the ordinates B, E, and C intersect MN, the distribution is represented by the blacked-in circle and the two circles of concentric rings. The inward surges of dispersal* from the selected focal points B and C meet at the colonisation point E, where an "equilibrium of distribution" is obtained. In the second generation, the blacked-in circle represents

*For purposes of explanation, radial dispersal is assumed.

the area where an "equilibrium of distribution" is obtained; outside this area the dispersal is only in an outward direction.

In the third generation, the overall area of distribution increases to nine times that of the first generation area and 2.25 times the area of influence after the second generation. This is the last generation in which the total area more than doubles the previous aggregate area; in each subsequent generation the proportionate addition to the previous aggregate area is progressively smaller. Using the five selected focal points where the ordinates A, B, E, C, D intersect MN, the distribution is represented by the blacked-in circle, the two circles of concentric rings, and the two blank circles in the first, second, and third generations or cycles, respectively. The blacked-in circle or first area now represents the area of "stable" distribution; the second area represents the area where an "equilibrium of distribution" is being obtained, and in the third area the dispersal is in an outward direction. In the first area, the reproductive power of the parasite will now be entirely expended in increasing its own population density.

To complete the discussion of the theoretical treatment of distribution, two other points should be considered. First, if the attraction of the host and of the habitat is optimum, there is a natural resistance to dispersal on the part of the parasite or predator. Second, after the entomophagous species has increased to an extent such that the environmental niche can no longer contain it, it will spread more rapidly and the time required for the destruction of the host population will be shortened. These two important considerations have received inadequate attention in colonisation programmes.

The above discussion explains part of the lag observed in the time required for the attainment of control after the initial introduction as compared with that required for the control of subsequent outbreaks. The size of the colony, the number and distribution of the colonies, and the rate of dispersal of the insect determine whether the equilibrium of distribution is established at a high or low level of density.

An insect with a slow rate of dispersal reaches an equilibrium of distribution rapidly, usually in the second parasite or host generation. The equilibrium in this case is at a high density level, probably with some degree of control already being obtained. The slower the rate of dispersal the quicker is the control attained. When control is rapid but confined to a small area, small colonies at frequent spatial intervals produce effective control over a large area in the shortest possible time. Commercial control by an introduced entomophagous insect with a slow dispersal rate is more quickly realised by an industrious colonisation programme to assist natural dispersal.

With an insect with a rapid rate of dispersal, the faster the rate the more widely is its attack upon the host population dispersed. An "equilibrium of distribution" is established at a low density level. Sometimes the density (as conditioned by colony size) is so low that the survival of the insect in the area is jeopardised. With a low initial density, it takes longer in terms of generations and time to obtain effective control than it does in the case of insects with a slow dispersal rate. Large colonies and sometimes secondary colonisation are necessary to effect establishment. Secondary liberations at the colony site will ensure efficient mating of widely dispersed sexes. Due to the wider dispersal, the effective control, when it becomes evident, appears simultaneously over a large area. Commercial control by an introduced entomophagous insect with a fast dispersal rate is more quickly realised by a few very large initial colonies followed by additional colonisation in the following generation, to counteract rapid dispersal. An effective colonisation programme can be assisted, where possible, by the release of mated insects ready to attack their hosts immediately.

This explanation substantiates Clausen's main thesis and is in agreement with Thompson's equations and postulates. It develops a refinement that answers, at

least in part, the objection that Clausen's conclusions do not explain the events that have been observed to occur. It should be remembered, however, that Thompson points out that a faster reaction in terms of time could be expected from the use of his formulae if a higher reproductive rate is used. The explanation offered in the above treatment substantiates his contention that complete control over a large area takes a good many years, especially in the case of an introduced insect with a slow rate of dispersal and unless the area is industriously colonised.

Clausen's and Thompson's papers either directly or indirectly suggest that some of the "mass production centers" are principally engaged in static instead of dynamic programmes. Clausen indicates that consideration should be given to his conclusions in revising current programmes and undertaking future efforts. Thompson states that more observation is required and that a proportionately greater effort should be expended to find out and understand what occurs in the field so that laboratory work may be conducted more efficiently. The writer has been associated with or has visited most of the large biological control centres. The one or two centres that make a reasonable effort to ascertain what occurs after the natural enemies are introduced have not made the information available. They should publish their information to assist those engaged in similar work. The institutions that have not collected such information should take suitable steps to secure it to justify their endeavours. It must be conceded that certain satisfactory accomplishments have been due to persistence, but at the same time much of the effort involved has been misdirected or wasted.

In developing the theoretical discussion of the theories of distribution and establishment, the examples and propositions cited by Thompson and Clausen have been continually reviewed. The area of agreement is substantially broad. The scope of Thompson's article covers the entire range of the time factor. Principally for this reason, it is not surprising that Thompson reached the conclusion that he did concerning Clausen's address. Clausen is primarily concerned with those species that have produced full commercial control. He suggests that the efficiency of these species is due to their special ability to put forth the additional effort to compensate for unfavourable environmental factors. This is not necessarily so, the reaction being rather an indication that optimum environmental conditions enable the insect to develop its potentialities to a higher degree. If conditions depart from the optimum we no longer have the fully effective parasite as defined by Clausen. In the opinion of the present writer, such ideal parasites do not exist.

Unless this theoretical treatment can be modified to conform to the characteristics of the partially effective species, it is of only academic interest. As might be expected, the performance of any particular parasite is variable. The ineffective species rarely performs above a minimum level, whereas the partially effective species ranges from this level upwards to occasional full effectiveness, depending upon the changing conditions and its own requirements. Several such species are as effective in the new environment as in the old, and are sometimes more so. In similar habitats, the optimum conditions that reflect the minimum environmental resistance necessary to demonstrate the full effectiveness of the species are only rarely encountered. If, however, the species is introduced into a superior environment, its effectiveness of control will be proportionately improved.

A brief review of the establishment in the north-eastern part of the United States of several natural enemies of the gipsy moth, *Porthetria dispar* (L.), will illustrate some of the additional complexities to be considered in relation to the time factor.

The gipsy moth has remarkable powers of increase under favourable conditions, both in Europe and the United States. When it encounters optimum conditions, the size of the egg clusters is usually double and sometimes from two-and-a-half

to three times the normal average size (400 eggs). The resurgence of the outbreak is usually of such intensity that the population exceeds the density that the environment is able to support. Rapidly following over-population, degeneration occurs and is accompanied by the rapid disappearance of the insect. By contrast with the uniform host population postulated in the theoretical treatment, we have in the gipsy moth periodical outbreaks. These outbreaks are local. They usually persist in various parts of any one area for three years and rarely four before disappearing. The fact that they are brought under control in a period of about three years may suggest that the controlling agencies are effective parasites in the sense of Clausen. The case therefore merits careful examination.

In Central Europe, between Transylvania and the Austrian Alps, the gipsy moth occurs in cyclical outbreaks of severe intensity. These outbreaks originate in the east and move progressively westward over a period of from three to four years. The cycles may occur as frequently as every seven years. Local outbreaks, usually arising from the general outbreak, are of more haphazard occurrence. The nuclei of local outbreaks are sometimes suppressed by biotic and physical agents, which results in the prolongation of the period between them.

In the north-eastern United States, there is a minimum period of from five to eight years between the epidemic cycles of the gipsy moth in New England. These outbreaks originate in the south and move progressively in a north-easterly direction over a period of years.

A great deal of work has been done on the ecology of the gipsy moth and its insect enemies in the United States, Europe, and Japan. In heavy outbreaks, its enemies are only rarely fully effective. The majority of the cases in which parasites have been more or less effective have occurred at relatively low population levels. The natural enemies often help to prevent a severe outbreak. The sequence of parasites, predators, and diseases operating in epidemic outbreaks of the gipsy moth is practically always classified as partially effective. Very rarely, the sequence, or individual members of it are described as fully effective. Howard and Fiske conclude that the predominant controlling factors of the gipsy moth are insect enemies at moderate host densities, disease when the infestation reaches a density beyond the normal level, and famine when it exceeds that which the environment can support.

Howard and Fiske (1911) and Burgess and Crossman (1929) cite many instances of effective control of the gipsy moth, both in Europe and Japan. The present writer made similar observations in Europe, usually in low or moderate population densities. Two of the larval parasites, *Apanteles liparidis* (Bouché) and *A. porthetriae* Mues., that showed outstanding promise in Europe, were not successfully established in the United States. The failure appears to have been due to lack of satisfactory alternate hosts. *A. liparidis* is very satisfactory in Europe when the gipsy moth occurs in association with *Dendrolimus pini* (L.). The identity of the alternate host of *A. porthetriae* in Europe was never satisfactorily determined. Obviously the result of the introduction of these two species of *Apanteles* was a matter of trial and error. The field recoveries in the season of liberation were excellent. In cases such as these, it is difficult to assess the value of the introductions in three years' time.

We shall now consider briefly some of the foreign insect enemies of the gipsy moth definitely established in the United States.

The two egg parasites, *Anastatus disparis* Ruschka and *Ooencyrtus kuvanae* (How.), are established. The available information suggests that *Anastatus* is a more effective parasite in the United States than in the countries from which it was imported. The parasitisation of an egg mass by it is entirely limited to the outer layer of eggs, *i.e.*, to not much more than one-third of the eggs. The maximum

parasitisation by *Ooencyrtus* is similarly circumscribed. Although an important egg parasite in the milder parts of southern New England, *Ooencyrtus* does not appear to be as effective as in Japan. Climatic factors appear to account for the difference. The habit of hibernation in the adult stage, which characterises *Ooencyrtus*, is not particularly suited to the harsh New England winter.

The initial liberations resulted in immediate establishment. Both parasites have a slow distribution rate, and their normal dispersal was supplemented by an industrious programme of colonisation and distribution. The tables and summaries supplied by Burgess and Crossman indicate that *Anastatus* was initially colonised in 1908 and, assisted by annual colonisation, reached its first peak of maximum effectiveness over the whole area in 1922 and 1923; *Ooencyrtus* was colonised in 1909, and over the years has given encouraging results only in the milder areas of New England. The steady increase of parasitism by *Anastatus* over a large area for a period of 14–15 years is of particular interest. If a parasite is as effective in its new environment as in its native habitat, the introduction must be considered successful even if the parasite is incapable of bringing about full commercial control. The maximum effectiveness of these two egg parasites obtainable within the span of three host generations is limited to 40 per cent. of the egg population; this represents 100 per cent. of the eggs that can be reached by *Anastatus* and *Ooencyrtus*. If the potential host population is below the limit of what the environment can support, the parasitism by these egg parasites is of control value. If, on the other hand, the parasite merely reduces the host population to the level at which the environment can support it, its value is questionable; without this control the host would have eliminated itself by exhausting the food supply. If the potential host population is above this level, the limited value of the parasitism is that of increasing the density of the parasite population available to attack the next generation, and owing to the slow dispersal rate of the parasite even this increase is of limited and doubtful value, because the outbreak of the gipsy moth will disappear either through starvation or degeneration.

Six larval parasites of the gipsy moth, comprising two species of parasitic Hymenoptera, *Apanteles melanoscelus* (Ratz.) and *Hyposoter disparis* Vier., and four species of parasitic Diptera, *Compsilura concinnata* (Mg.), *Exorista larvarum* (L.), *Sturmia scutellata* (R.-D.) and *Parasetigena silvestris* (R.-D.), are established in the north-eastern United States.

On the whole, *Apanteles melanoscelus*, with two annual generations, is as effective in its new habitat as in its old, so its introduction must be considered a success. It was first colonised in 1911, and was recovered sparingly at first but in increasing numbers in each successive year until it became noticeably abundant five years later, in 1916. There was a natural spread in a north-easterly direction indicating wind dispersal of either the adult parasites or small parasitised gipsy moth larvae. Apart from suitable host numbers, its limiting factors appear to be climate and the effect of a great variety of hyperparasites, especially on the winter cocoons. Despite these limiting factors it is often fairly abundant in limited areas, 20 to 30 per cent. parasitism being not uncommon. Nevertheless, *A. melanoscelus* seldom qualifies as an effective parasite. With a single exception in Italy, *Hyposoter disparis* has much the same effect in New England as in Europe. It is not important as a controlling agent, appearing to be poorly adapted to the gipsy moth as a host. Muesebeck and Parker (1933) point out that parasitism is apparently heavier in dense woodland than in open growth or outer edges of woodland areas. Although it was recovered in the year following its liberation in 1912, it has never been collected in sufficient numbers to permit its slow dispersal to be supplemented adequately through colonisation. Climate and hyperparasitism have probably contributed their share to its ineffectiveness. One could not expect that this parasite would be more effective in its new environment, but its limitations were better defined after

the lapse of a twenty-year period than in the first few years after it was located in Europe. It is evident that there is little likelihood of either *Apanteles melanoscelus* or *Hyposoter disparis* having more than a limited annual effect on the gipsy-moth population, yet they are capable of demonstrating their capabilities within one host generation.

There are good reasons for thinking that *Compsilura concinnata* is a more valuable parasite in its new environment than in Europe. The greatest contributing factor appears to be the larger number of alternate hosts in North America. The species was successfully colonised in Canada from New England, becoming established on the west coast, in British Columbia, and on the east coast, in Quebec. In the north-eastern United States and Canada, its range extends far beyond the areas infested by the gipsy moth, brown-tail moth and satin moth. It is a valuable controlling agent of these three pests as well as other insects. Its value depends upon the abundance of suitable hibernating hosts in the infested areas. *Compsilura* is repeatedly more effective against the brown-tail moth and the satin moth than against the gipsy moth; this is especially noticeable in areas where they occur in association. As the brown-tail and satin moths hibernate as larvae, they are available for spring attack much earlier than the gipsy-moth larvae which reach the adequate size for successful attack later in the spring. *Compsilura* is an efficient parasite in light infestations of the gipsy moth, especially in areas where plenty of hibernating hosts are available. In the central hardwood region of southern New England and in portions of the white-pine region, in very light infestations of the gipsy moth, parasitism by *Compsilura* often ranges to 75 per cent. or more. In heavier infestations although the *Compsilura* population in a given area may actually be higher, the effectiveness of the attack is dissipated in the large host population, and any value that might result in the next generation of the gipsy moth from a concentration of a large *Compsilura* population is nullified by the necessity to pass one or more generations on alternate hosts.

Compsilura was first liberated in 1906 in very small numbers. It was recovered in 1909 and its dispersal thereafter was extremely rapid. Records indicate that it has spread as much as 25 miles in a single season. The successful introduction of it into North America has certainly provided a bewildering array of complexities in respect of the time factor in biological control. However, the benefit derived from this parasite is worth all the time and effort that have been expended over a period of years.

Exorista larvarum is another multibrooded Larvaevorid that has been successfully introduced into the north-eastern United States. In Europe it has been recorded from many hosts, including the gipsy moth, the satin moth and the brown-tail moth; its racial counterpart has been recorded from the gipsy moth in Japan. In New England it has been recovered from the satin moth (1940), the gipsy moth (1941) and the brown-tail moth (1941). This parasite was introduced from Europe in small numbers and liberated during the years 1906-1911. There is no information to indicate that it was established from these early releases. During the more recent importation work between the years 1925 and 1932, it was received in large numbers from Europe and widely colonised in New England. Burgess and Crossman (1929) consider that during the summers of 1926 and 1927 it was colonised much more satisfactorily than at any previous time. Taxonomically this species has been confused with the nearctic *Exorista mella* (Walker), which has apparently been reared occasionally from the three above-mentioned hosts.

The first authenticated recovery of *E. larvarum* was made in 1940 from a collection of seven larvae and three pupae of the satin moth made at Waterbury, Connecticut. This collection produced 14 *Exorista larvarum*, five *Compsilura concinnata*, and one male and one female satin moth. The determination of *Exorista larvarum* was

based on morphological differences in all three larval instars between *E. mella* and *E. larvarum* discovered by the late R. T. Webber, the late T. H. Jones, and the writer. Past records indicate that the American species is rarely reared from the gipsy, brown-tail or satin moth.

While the establishment and distribution of *Parasetigena silvestris* in New England were being studied in 1941, over 100 puparia of *Exorista larvarum* were recovered from 11 of 69 townships where gipsy-moth larval collections were made, *i.e.*, Standish, Maine; Northwood and West Concord, New Hampshire; and Salisbury, Newbury; W. Newbury, Lawrence, Wenham, Andover, Ipswich and Berkley, Massachusetts. At Salisbury, the parasitism of the gipsy moth by *E. larvarum* reached 9 per cent. Also in 1941, *E. larvarum* was recovered from collections of brown-tail larvae made at Stratham and Rye, New Hampshire, by W. S. McLeod of the Imperial Parasite Service (Commonwealth Institute of Biological Control). The recovery of such numbers of puparia confirms the identity of the parasite as *E. larvarum*. Furthermore, *E. larvarum* invariably forms its puparium outside the host larval skin, whereas *E. mella* is apt to form its puparium inside it or only partially emerged from it.

As *E. larvarum* is multibrooded, its abundance in any particular locality will be somewhat dependent upon the abundance of satisfactory hibernating hosts. The recoveries from the satin moth at Waterbury, Conn., and the gipsy moth at Salisbury, Mass., indicate that it can be a valuable addition to the parasites already established in the United States and Canada. The case of *E. larvarum* shows that a parasite destined to become an important controlling factor may require much more than three years to manifest its capabilities, and in this respect *E. larvarum* contrasts with *C. concinnata* which gave promise of usefulness very soon after it was introduced. There is little likelihood of either *Compsilura concinnata* or *E. larvarum* having more than a limited annual effect on any population of the satin, gipsy or brown-tail moth, but in very light infestations, under certain conditions, effective control can be anticipated. In light to moderate infestations, both species can be expected to exert a partial check. In heavy infestations, the parasite population would be thinly distributed, making the ratio of control low or relatively useless.

Sturmia scutellata appears to be a more valuable parasite of the gipsy moth in the north-eastern United States than in Europe. In 1929, Burgess and Crossman ranked it among the best enemies of the gipsy moth which had been successfully established. In Europe, *Hemipenthes morio* (L.) is an important parasite of *S. scutellata*, and in the United States, *Brachymeria compsilurae* (Cwfd.) is a very destructive enemy of it. In Europe, *S. scutellata* faces more competition from the other larval parasites. The establishment of *Exorista larvarum* and *Parasetigena silvestris* will tend to offset somewhat any advantage that lack of competition afforded *S. scutellata* earlier in the United States.

In 1907, a few *Sturmia* were liberated under unfavourable conditions. From 1908 to 1911, 5,372 adults of foreign origin were liberated. The first encouraging recovery of this parasite was made in 1916, and its numbers steadily increased until, in 1923, the average parasitism of all the recovery collections from the whole area indicated that nearly half of the female pupae were parasitised by it. *Sturmia* lays several thousand eggs and disperses rapidly. It did not need the assistance of a widespread programme of artificial colonisation as did some of the other parasites.

At times the effect of *Sturmia scutellata* is indeed impressive. Burgess and Crossman emphasised its importance in the lightly infested areas. Observations by the writer showed that at the start of oviposition there is a tendency for the eggs to be restricted to the immediate area of a leaf where the gipsy moth larva is feeding. Later, they are indiscriminately deposited on leaf surfaces throughout the woodland. Although a few puparia are obtained from full grown larvae, the majority of the parasites emerge from the pupae. The maximum value of *S. scutellata* can only be

ascertained from larvae collected after they have stopped feeding or from collections of freshly formed pupae. Two or three times as many *S. scutellata* are obtained from collections of female pupae as from male pupae, and the percentage parasitised is higher in the former. There is a simple explanation for this. The female gipsy-moth larva has six instars, one more than the male. In the sixth-instar, the larva consumes considerably more foliage than in all the other instars together, and consequently ingests a proportionately greater number of *Sturmia* eggs. Although there are additional reasons why the proportional consumption of eggs should be greater, it can safely be assumed that the female larvae consume at least two-thirds of the eggs. Moreover, the larger female larvae are more prone to superparasitism and frequently produce two puparia.

Even in the lighter infestations, there is a good chance of a number of *Sturmia scutellata* eggs being consumed; as few as 200 females are capable of depositing at least a million eggs. The species is most effective in outbreaks where there is complete defoliation without actual starvation of the gipsy moth. Under these conditions all the *S. scutellata* eggs deposited on the leaves are ingested. These special conditions seldom occur, however, so that this parasite can rarely display its maximum effectiveness. Usually, under more severe conditions, starvation, disease and degeneration of the population occur, and the part that *S. scutellata* plays in the control of such outbreaks is of little economic importance.

Parasetigena silvestris is one of the outstanding larval parasites in Central Europe, commonly destroying from 30 to 35 per cent. of the gipsy-moth larvae occurring under epidemic conditions. This destruction normally takes place in the second or third year of an outbreak. This occurs despite the fact that the species is seldom encountered between outbreaks. *P. silvestris* was imported into the United States during the periods 1907-1909 and 1923-1932 under the names *Parasetigena segregata* of authors, not Rondani, and *Phorocera agilis* of authors, not Robineau-Desvoidy. Prior to 1927, liberations of it were made under conditions that appear to have precluded its successful establishment. In 1927, the first successful liberation was made, at Boxford, Massachusetts. In 1928, secondary liberations were made in Boxford and the nearby area of Rowley, Mass. Field recoveries were made in 1927 and 1928 at Boxford, and recoveries indicating establishment in 1929 at Boxford and Rowley. From 1924 to 1933, inclusive, liberations were made in 22 localities, and prior to 1941, recoveries indicating establishment were made at four of the colony-site areas. The first three successful establishments of this parasite followed liberations in two successive generations in the same area. In the fourth recorded establishment, 11 years after liberation, there exists the possibility of natural spread from adjacent areas; at this place the policy of double liberation was not adopted. In 1932, it was recovered from two localities where it had not been liberated, Ipswich and Wilmington, Mass. It is a strong flier and has been spreading since 1934 without any supplementary liberations in the area. In 1941, when a rather large-scale check was made on its distribution, it was recovered from 35 localities indicating that it was distributed along the Atlantic seaboard in New England, extending roughly from Sebago, Maine, in the north to Ossipee, New Hampshire, and Lunenburg, Massachusetts, in the west and to Thompson, Connecticut, in the south. Twenty-five of the recoveries were new distribution records. Most of them were from the Essex-County area in Massachusetts which indicated the development of a density only slightly beyond that of an "equilibrium of distribution" around the Boxford, Rowley and Ipswich area in the second epidemic cycle of the gipsy moth after liberation of the parasite. In 1942, W. F. Sellers and W. S. McLeod recovered *P. silvestris* from gipsy-moth larvae collected in Rehoboth and Attleboro, Mass., Nottingham, Durham, and Auburn, N.H., and the Sebago Lake area in Maine. Of these six localities, the first and last are original colony sites and the others are new distribution records. The recoveries were made while *Compsilura concinnata* was being collected for shipment to the Union of South Africa.

It is not unlikely that in the course of time *Parasetigena silvestris* will prove as effective in its new environment as in Europe. In Europe, it is also one of the principal parasites of the nun moth, *Lymantria monacha* (L.). It will be interesting to watch its activities in the United States where it has only one of these hosts available.

In Europe, the writer has seen incipient outbreaks of the gipsy moth eliminated by *Parasetigena silvestris*, with the help of other parasites. Burgess and Crossman state that in Europe "in some cases where several gipsy-moth infestations have been nearly wiped out this parasite has been largely responsible, although several other enemies of the gipsy moth had a part in this control, especially *A. portheiriae* and *S. scutellata*". In Europe, *P. silvestris* has repeatedly been recorded as the predominating dipterous parasite in large-scale outbreaks of the gipsy moth, but its value, like that of *S. scutellata*, diminishes if the gipsy-moth infestation exceeds the capability of the environment to support it, but in the earlier stages of an outbreak, these two parasites increase in value with the increasing gipsy-moth infestation and both reach their maximum effectiveness during the third year of the outbreak at about the time when the degeneration occurs. They differ, however, in that whereas *S. scutellata* became established quickly in the United States, the establishment of *P. silvestris* was not achieved until certain essential requirements had been satisfied.

Calosoma sycophanta (L.), a Carabid predator of the larvae and pupae of the gipsy moth in Europe, is established in the United States. It increases as the gipsy moth increases and exerts its maximum effect at about the time when the outbreak reaches its peak. The beetle has demonstrated its effect at the end of heavy outbreaks both in Europe and the United States.

One of the most important controlling agencies in heavy outbreaks is the virus disease, *Borrelina reprimens* Holmes, which causes polyhedrosis of the gipsy-moth larvae. Examination of old records leads to the impression that this wilt disease was accidentally introduced into New England with the early parasite importations. It reaches its maximum virulence in heavy and degenerating outbreaks of the gipsy moth. Now that more is known about the chemical properties of this virus, the deliberate utilisation of it should be attempted. The objective would be to create a maximum degree of contamination in incipient or very light infestations of the gipsy moth. The virus would have all the attributes of the perfect insecticide in that the gipsy moth is the only organism upon which it is lethal.

This discussion of the results of the importation into the United States of natural enemies of the gipsy moth has been presented to augment, from practical experience our understanding of the time factor in relation to a number of partially effective parasites (in Clausen's sense). In practically every case, the parasite has proved to be as effective in its new environment as in the old or even more so. Although seldom qualifying as effective controlling agents as defined by Clausen, most of the species discussed attain their maximum effectiveness in the third year of the epidemic outbreak. Some of them are so limited in their activities that they can exert only a limited annual effect on the outbreak, and this category includes those that are the most efficient controlling agencies in the lighter infestations. Consideration of the results of the introduction of the various controlling agents of the gipsy moth in the United States indicates that the establishment of an "equilibrium of distribution", after which they can exert their full pressure, takes longer than three years; in fact, it appears to take place in the second cyclic outbreak after liberation.

At the present time, the degree of control of the gipsy moth achieved in the north-eastern United States does not differ greatly from that which occurs in Central Europe.

Conclusions.

Clausen's views concerning the time that is required for imported parasites and predators to exert their full pressure are supported by the observed facts, especially

when the natural enemies are fully effective. During the process of establishment, there is a time lag that does not occur after an equilibrium of distribution has been achieved. The principal factors involved are the time necessary for the attainment of the equilibrium of distribution and the rate of dispersal relative to the building-up of a controlling density. Parasites with slow dispersal rates bring about effective control more quickly, but in a proportionately smaller area, than those with fast dispersal rates. The time necessary for fully effective control varies from three host generations to a three-year period.

When these considerations are applied to the requirement of the partially effective parasites, the time element is proportionately extended beyond the three-year period. Many partially effective enemies are as effective in the new environment as in the old, and the time involved, though much more variable than in the case of the fully effective enemies, is sometimes not much longer. In general, however, an enemy that is to become useful but not completely effective does not show its value within three years; consequently the adoption of Clausen's conclusions involves a danger of discontinuing programmes prematurely.

Clausen maintains that a parasite that is fully effective appears to be able to put forth an additional effort to compensate for unfavourable environmental factors. It is difficult to substantiate this claim. The natural control of organisms is basically due to the intrinsic limitations of the organisms themselves. In the more or less partially effective parasites of the gipsy moth, the limiting factors appear more often than not to be inherent in the host. In both instances the results observed indicate that the environmental conditions help or hinder the insects in developing their potentialities.

Thompson has persistently drawn attention to "the woefully inadequate character of our information on the increase and spread of introduced parasites". This challenge must be met. In many biological-control projects, control is achieved without being foreseen or understood. Work in this field is not satisfactory unless careful ecological studies are conducted both before and after the establishment of the natural enemies. The whole field needs to be critically reviewed and frequently re-examined in the future to utilise new techniques and evidence and also to improve the quality of practical programme and investigational research.

Summary.

C. P. Clausen's and W. R. Thompson's concepts on the time factor in biological control are compared with the writer's experience. All three viewpoints emphasise the influence of environment correlated with time. As Thompson points out, we are almost always forced to ascribe the limitations of the numbers of pests to a combination of factors, biotic and physical, varying from time to time and from point to point. Clausen's views concerning the maximum time (three host generations or three years) necessary for an introduced parasite to demonstrate that it will be fully effective are supported by the observed facts. There is associated with the initial establishment of introduced parasites, however, a time lag that is not evident in the control of subsequent outbreaks. The principal factors are the attainment of an "equilibrium of distribution" and the rate of dispersal in building up a controlling density. When effective biological control can be anticipated, it is characteristic that marked outbreaks are brought rapidly under control, and the pest population then oscillates at a relatively low level. Several examples of fully effective parasites and their limitations are discussed which indicate that the value of a parasite or predator should be regarded not as a constant but rather as a variable.

An equilibrium of distribution is obtained when there is no decrease in the density of a parasite due to outward dispersal into the uncolonised area; the forward and backward surges of dispersal offset each other, producing a minimum uniformity of

distribution independently of other influences. Parasites with slow dispersal rates produce an equilibrium of distribution and effective control more rapidly, but in a proportionately smaller area, than those with fast dispersal rates.

When these considerations are applied to the introduced, partially effective parasites of the gipsy moth, the time element is seen to be proportionately extended beyond the three-year period. After establishment, however, the effectiveness of these parasites is conditioned by the normal duration of the heavy outbreaks of the gipsy moth which rarely exceeds three years. Many of these enemies are as effective in the new environment as in the old. The degree of control of the gipsy moth achieved in the north-eastern United States does not differ greatly from that which occurs in central Europe. Examination of this relationship indicates that an enemy that is to become useful, but not completely effective, does not show its value within three years; consequently the adoption of Clausen's conclusions involves a danger of discontinuing programmes prematurely.

Acknowledgements.

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References.

- BERGOLD, G. H. (1947). Die Isolierung des Polyeder-Virus und die Natur der Polyeder.—*Z. Naturf.*, **2b**, pp. 122–143.
- BURGESS, A. F. & CROSSMAN, S. S. (1929). Imported insect enemies of the Gipsy Moth and the Brown-tail Moth.—*Tech. Bull. U.S. Dep. Agric.*, no. 86, 147 pp.
- CLAUSEN, C. P. (1951). The time factor in biological control.—*J. econ. Ent.*, **44**, pp. 1–9.
- DARWIN, C. (1859). *On the origin of species*. . . .—London.
- HOWARD, L. O. & FISKE, W. F. (1911). The importation into the United States of the parasites of the Gipsy Moth and the Brown-tail Moth. . . .—*Bull. U.S. Bur. Ent.*, no. 91, 344 pp.
- MUESEBECK, C. F. W. & PARKER, D. L. (1933). *Hyposoter disparis* Viereck, an introduced Ichneumonid parasite of the Gipsy Moth.—*J. agric. Res.*, **46**, pp. 335–347.
- SOLOMON, M. E. (1949). The natural control of animal populations.—*J. Anim. Ecol.*, **18**, pp. 1–35.
- STEINHAUS, E. A. (1949). Nomenclature and classification of insect viruses.—*Bact. Rev.*, **13**, pp. 203–223.
- THOMPSON, W. R. (1929). On natural control.—*Parasitology*, **21**, pp. 269–281.
- THOMPSON, W. R. (1951). The time factor in biological control.—*Canad. Ent.*, **83**, pp. 230–240.
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OBSERVATIONS ON THE ENTRY OF DUSTS INTO THE RESPIRATORY SYSTEM OF THE ADULT WORKER HONEY BEE, *APIS MELLIFERA* L.

By Joan U. CONNELL & G. D. GLYNNE JONES.

Seale-Hayne Agricultural College, Newton Abbot.

(PLATE VI.)

The entry of dusts into the respiratory system of an insect has been shown by Webb (1945a) to depend on (a) whether the structure of the inspiratory spiracles will allow the passage of a fine dust into the tracheal system and (b) whether the insect breathes by tracheal ventilation or by diffusion.

As part of a general study of the effect of plant protective chemicals on the honey bee, *Apis mellifera* L., an attempt was made to determine whether any structures in or associated with the spiracles can function to remove dust particles from inspiratory air streams. Snodgrass (1925) in his description of the structure of the spiracles indicated the presence of hairs in the atrium of the abdominal spiracles but made no mention of their function.

The general position of the three thoracic and seven abdominal spiracles is indicated in fig. 1. Internally, parts of the tracheal system are expanded into thin-walled air sacs.

Significance of the Hairs surrounding the Spiracles.

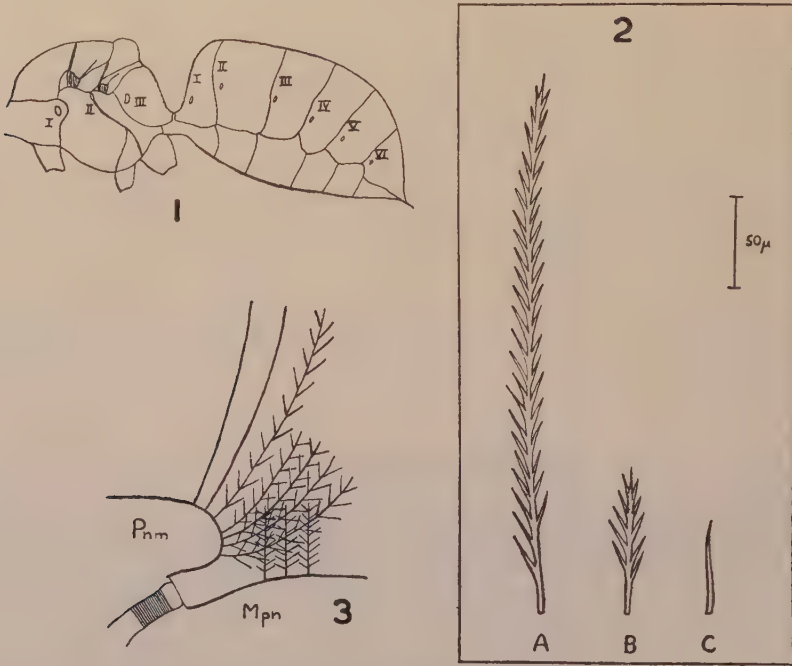
The honey bee possesses a dense covering of varying types of hairs, which play an important part in the collection of pollen. These hairs are not distributed uniformly over the surface of the cuticle, some areas being apparently better covered than others. Examination of the hairs around the spiracles showed the presence of three types similar to those found over the general body surface, namely long compound, short compound, and simple hairs (fig. 2). The hairs arising from an area of cuticle closely surrounding each spiracle were examined under the microscope with a view to ascertaining whether there was any peculiar arrangement present in this region, which might tend to prevent the entry of particles into the spiracles.

First thoracic spiracle.

The first thoracic spiracle (fig. 3) is situated in the intersegmental membrane, concealed beneath a flap-like chitinous lobe which projects back from the rear margin of the pronotum. It is protected by a dense fringe of long compound hairs, which have their origin on the pronotum (Pnm). These hairs are arranged in two layers, an outer layer of long hairs (type A), and an inner layer arising from the edge of the pronotum, of shorter hairs (type B). In addition there is a layer of hairs (of the same general structure as type B hairs but twice their length) arising from the cuticle of the mesopleuron (Mpn) beneath the pronotum flap, which project vertically upwards and interlock with the long hairs of the pronotum. In this way a meshwork of protective hairs is formed around the region of the spiracular opening. Measurements of the average distance apart of the bases of these hairs indicate that spherical particles greater than 20 microns in diameter will be filtered out before they can reach the cuticle. A large percentage of smaller particles may be also held back by the side branches of the hairs, which further limit the space available for penetration.

Second thoracic spiracle.

The second thoracic spiracle is very minute, and is situated below the wing base, in the membrane between the upper edges of the mesopleuron and the metapleuron. The aperture itself is exceedingly small and is sunk below two overlapping chitinous rims. Arising from these rims and the surrounding cuticle are long compound hairs, which form a fringe along the suture between the meso- and metapleuron. These hairs are not vertical, but tend to curve inwards over the spiracular opening and meet in the mid-line. Measurements showed that the hairs would have a very similar filtering action to those of the first thoracic spiracle.



Figs. 1-3.—1. Position of spiracles in the adult worker honey bee.
 2. Three main types of hairs found on body surface.
 3. Diagrammatic representation of arrangement of hairs at entry of first thoracic spiracle.

Third thoracic spiracle (Spiracle of the propodeum).

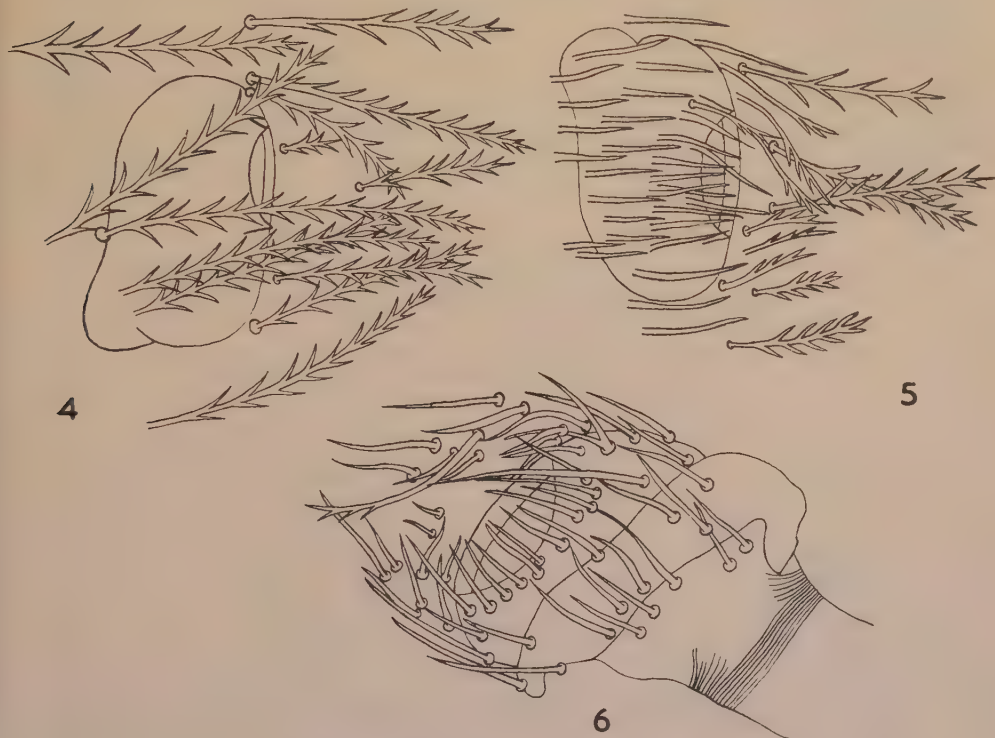
The third thoracic spiracle is the largest and lies fully exposed on the side of the propodeum. Even so, it has a less dense distribution of hairs around it than the other thoracic spiracles. Measurements indicated that particles less than 50 microns could penetrate the hairs though a percentage of these would again be held by the side branches.

Abdominal spiracles.

The abdominal spiracles are found in the lower parts of the tergal plates, with the exception of the last, which is not visible externally, being closely associated with the base of the sting.

The opening of the first abdominal spiracle faces in an antero-lateral direction, and is well protected by upright hairs, chiefly of type A. The distance apart of the hairs was found to range from 28-30 microns.

The openings of the next five spiracles are sunk slightly below the level of the cuticle and face in a posterior direction. The arrangement of hairs around the second abdominal spiracle (fig. 4) is very similar to that of the third thoracic spiracle. The hairs, which are all of type A, project almost vertically from the surface of the cuticle and slant slightly backwards, at an average distance apart of 50 microns.



Figs. 4-6.—Arrangement of hairs around orifices of second, fourth and sixth abdominal spiracles.

The hairs of type A around the third abdominal spiracle (Pl. VI, fig. 1) are considerably reduced in number, the spiracle being surrounded chiefly by hairs of type B, which point backwards and lie parallel with the surface of the cuticle. These hairs are approximately 18 microns apart. The distribution of hairs around the fourth and fifth abdominal spiracles are very similar to one another (fig. 5). The number of short compound and simple hairs is greatly increased with only one or two scattered hairs of type A. The sixth abdominal spiracle (fig. 6) is surrounded by simple hairs only, and the concealed seventh spiracle is entirely devoid of hairs.

From the foregoing description of the hairs surrounding the openings of the spiracles, it can be seen that there is a wide variation in both the density and type of hair present in the honey bee. Around the abdominal spiracles this is most marked. Proceeding from the first to the sixth, there is a progressive decrease in the numbers of type A hairs and a progressive increase in type B, the latter eventually giving way to a dominance of type C. Under field conditions the hairs, particularly types A and B, would probably serve to prevent pollen from clogging the spiracular openings, and this is least likely to occur at the posterior end of the bee. It is also considered likely that the hairs would act as filters to dust particles in an inspiratory air stream.

These general observations were tested by exposing flying bees to dust clouds of known particle size. A china clay dust with particles ranging from 30–54 microns was used and the results obtained from an examination of the spiracular openings with a low power binocular microscope for the presence or absence of dust particles were compared against the anticipated effects deduced from the previous observations and measurements (Table I). It can be seen that with the exception of the third thoracic and the fifth and sixth abdominal spiracles, the deduced and actual results obtained for the penetration of dust particles through the external spiracular hairs were found to agree. The discrepancy is possibly explained, in the case of the third thoracic spiracle, by its position, relative to the direction of air streams which the thorax will encounter during flight through dust clouds. The penetration of dust particles through the spiracular hairs of the fifth and sixth abdominal spiracles is probably due to the nature of the hairs themselves, which are chiefly unbranched simple hairs, of type C.

TABLE I.
Comparison of the deduced and actual penetration of dust particles (30–54 μ diameter) through the hairs which surround the spiracular openings.

			Expected result from hair density	Actual result from dusting
Thorax				
Spiracle I	—	—
Spiracle II	—	—
Spiracle III	+	—
Abdomen				
Spiracle I	—	—
Spiracle II	+	+
Spiracle III	—	—
Spiracle IV	—	—
Spiracle V	—	+
Spiracle VI	—	+

+ or — indicate presence or absence of dust particles.

Internal Examinations of Spiracles.

Method and materials.

The internal structure of the abdominal spiracles was determined from sections cut transversely through the honey bee. Adult bees at least five days old were used, since there is some evidence for supposing that the spiracular structure of recently emerged bees differs slightly from that of the adults. The use of adult bees made sectioning extremely difficult, since the chitinous exoskeleton, even when softened, presented a very hard surface to the microtome knife edge.

A sledge microtome was used ; this held the embedded material rigid, and cut sections horizontally, with a slicing movement. Sectioning by this means was tedious, as each section had to be lifted off the knife separately. To prevent curling, a piece of stiff paper cut to the size of the block and well moistened, was placed on the block, thereby inducing the section to stick to the paper in preference to the knife.

Before fixing and embedding, the bees were cut in a vertical longitudinal plane, to facilitate the entry of fixing fluid and paraffin wax. They were then fixed in Carnoy's fluid for 48 to 72 hours, dehydrated and cleared in cedar-wood oil for 24 hours. The bees were transferred directly into paraffin wax having a melting point of 52°C. ; two changes of wax were used, for 30–45 minutes each. Wax blocks were

made in the usual way. Sections were cut approximately 10–20 μ thick, and mounted in Canada balsam, without staining.

Results.

Sections through the abdominal spiracles showed the presence of hairs, projecting inwards from the outer atrium wall, into the cavity of the atrium. The hairs were arranged in such a way that they could provide a filtering mechanism for particles present in an incoming air stream which might otherwise penetrate into the connecting trachea. The average length of these hairs was found to be 12 microns. There were no equivalent hairs in the thoracic spiracles.

As the size of dust particle entering the spiracle will also be limited by the size of the elliptical spiracular opening, measurements of the length and breadth of the latter were made (Table II). The smallest and largest are the second and third spiracles, respectively, found on the thorax; the first thoracic spiracle is also larger than the majority of the abdominal spiracles. The abdominal spiracular openings are shown to be uniform in size with the exception of the first and last.

TABLE II.
Measurement of spiracular openings.

	Length (Microns)	Breadth* (Microns)
Thorax		
Spiracle I	100	57
Spiracle II	13	4
Spiracle III	238	61
Abdomen		
Spiracle I	110	22
Spiracle II	77	31
Spiracle III	76	25
Spiracle IV	74	25
Spiracle V	82	22
Spiracle VI	78	28
Spiracle VII	121	53

*This measurement represents the maximum breadth.

Mechanism of the Respiratory System.

In order to proceed with this study, it was found necessary to consider the actual mechanism of tracheal ventilation in the honey bee. No satisfactory account was found in the literature, though the presence of valves as described by Snodgrass (1925) and Wohlgemuth (1929) indicates that tracheal ventilation does occur, and observational experiments were undertaken in an attempt to obtain further information.

It would appear that three possible mechanisms of tracheal ventilation could occur:—

- (a) Inspiration through the abdominal spiracles, expiration through the thoracic spiracles, as in the sheep ked (Webb, 1945b).
- (b) Inspiration through the thoracic spiracles, expiration through the abdominal spiracles.
- (c) Both thoracic and abdominal spiracles being capable of serving either function.*

*Wohlgemuth (1929) made observations on the behaviour of air bubbles trapped in the spiracular region of bees immersed in water, and also on the rate of paralysis when the thorax and abdomen respectively were placed in chloroform vapour. He concluded that all spiracles had an inspiratory function but only the thoracic spiracles were expiratory. No great importance can be attached to these conclusions.

In either (a) or (b) there would always be a directed flow of air through the propodeum. An attempt was made using a modification of the apparatus used by Fraenkel (1932) to detect the presence of such an air flow (fig. 7). A small hole corresponding in size to the narrowest part of the bee's propodeum was cut in an elastic membrane. The hole was enlarged by stretching the membrane, the abdomen of the bee passed through and the membrane relaxed at the propodeum. The membrane was then firmly attached to tube X and sealed off around the propodeum with a low melting point wax. Chambers A and B were checked for airtightness and the whole apparatus was placed in a constant temperature cabinet. A small drop of manometer fluid was introduced into each capillary tube, a and b, and the stop cocks closed. Observations for over two hours failed to reveal any volume changes in either chamber, though the indicator fluid did exhibit regular pulsing movements of small amplitude in both capillary tubes. There was no evidence of any directional airflow through the propodeum but it was possible that such a method of mounting the bee constricted the propodeum.

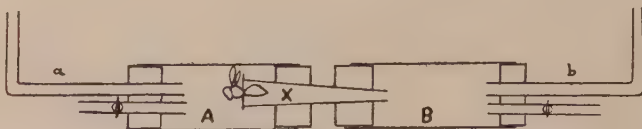


Fig. 7.—Diagram of apparatus used in tracheal ventilation experiment.

Further confirmatory evidence was obtained as follows :—

Two samples of living bees were taken ; the bees of one sample were ligatured at the propodeum, and the others had their abdomens removed. Apart from an increase in the activity of the bees with no abdomens, nothing unusual was observed. Both sets of bees continued living for a considerable period of time. If the ligatured bees were dependent on their abdominal spiracles for a supply of inspired air, then their early death would be expected. This did not occur, and it is apparent that the thoracic spiracles are capable of satisfying the oxygen requirements of that region of the bees.

Bees were filmed with a ciné camera whilst hovering over a dish of syrup at exposures of 1/120 second and a film speed of 64 frames per second. Measurements of the length of the abdomen on consecutive frames failed to show any changes in length which would indicate rhythmical movements. As the abdomen appeared in clear focus, it was assumed that it was not exhibiting high speed vibrations at this exposure.

The results of these two tests and photographic observations when considered collectively tended to eliminate possibilities (a) and (b), and it would therefore seem unlikely that the pumping action of the abdomen, which is so often visible in adult bees, is used for the forcing of a stream of air into the thorax. Later experiments with dusts showed that both the thoracic and abdominal spiracles can be inspiratory, and we must, therefore, conclude that contention (c) is the most likely. Hamilton (1937) showed that in the locust certain abdominal spiracles only had an inspiratory function, and the others expiratory. It is considered unlikely that this occurs in the honey bee as all abdominal spiracles open into the large paired abdominal air sacs.

Entry of Dusts into the Respiratory System.

Previous observations have shown that it is theoretically possible for dusts of particle size less than 25 microns to enter all the spiracles with the exception of the small second thoracic spiracle.

Bees which had been exposed to clouds of different dusts were then examined in detail in order to ascertain the extent of penetration into the tracheae and air sacs.

Materials and method.

Preliminary experiments with different dusts showed that only black particles were easily visible in the air sacs and tracheae. The dusts finally selected for use were powdered charcoal and cuprous cyanide (Hamilton, 1937), the latter showing up, on exposure to hydrogen sulphide, as cuprous sulphide, which is black. The dusts were put through a B.S.S.300 mesh sieve and microscopic examination showed them to contain particles in the range 2–40 microns, a large proportion of particles being less than 20 microns.

Fifty bees were placed in a large metal container of ten litres capacity. A jet of compressed air was forced across a quantity of the dust and this generated a dust cloud within the chamber. The turbulence this induced ensured active flight, and small amounts of carbon dioxide were introduced to stimulate active respiration. After treatment for ten minutes, the bees were killed slowly with hydrogen sulphide.

The respiratory system of the bees was then very carefully dissected under water. Where cuprous cyanide had been used, no particles of this substance were found in any of the air sacs or tracheae. In the case of charcoal, however, particles were detected high up in the main tracheal trunk, leading from the first thoracic spiracle. This was probably due to the extra fineness of the charcoal particles, which tended to form aggregates after entry. The diameter of the smallest charcoal particles averaged less than 5 microns.

A further sample of bees, which had been dusted with cuprous cyanide, were then carefully washed to remove all dust adhering to the external surface of the cuticle, and prepared for sectioning as previously described. The serial sections were only successfully obtained for the first four abdominal spiracles and, in these, some dust particles were seen to have been trapped by the hairs within the atrium of the spiracle (Pl. VI, figs. 2 and 3). The range of particle sizes (diameter in microns) of cuprous sulphide found within three abdominal spiracles was as follows:—20, 15, 14·5, 11·6, 8·5, 8·5, 8·3, 8·3, 8·3, 7·5, 7·0, 7·0, 6·6, 6·2, 4·1, 4·1, <4·1.

These figures show that particles of cuprous cyanide ranging from 4 to 20 microns in diameter were being filtered out of the incoming air streams and a typical section through a spiracular atrium and connecting trachea (Pl. VI, fig. 3) indicates that it is very unlikely that any of the particles were actually entering the trachea. It must be mentioned, however, that the accurate measurements of particles less than 4 microns in diameter was difficult, although they could be easily seen under the microscope.

Discussion.

The observations and experiments have indicated that in the respiratory system of the adult worker honey bee there is an efficient filtering mechanism which will remove particles greater than 4 microns from the incoming air streams. Some initial filtration occurs in the surface hairs which surround the spiracular orifices and the latter hairs would apparently normally function to prevent this opening from becoming clogged with pollen. Further filtration is brought about by the size of the spiracular opening, and also by the hairs within the vestibule. No dust particles were found in any part of the tracheae or air sacs with the exception of the first thoracic tracheal trunk. Here only the lighter and finer of the two dusts used was found.

No conclusive information was obtained on the mechanism of tracheal ventilation, though it was apparent that all spiracles are capable of transmitting inspiratory air streams. This is of interest as it indicates that the abdominal movements do not supply the large oxygen requirements of the thoracic muscles when the insect

is in flight. It would appear, therefore, that the thoracic tracheal system is ventilated by the direct action of muscles associated with the wings.

Whilst little information is available on the range of particle sizes present in the common types of inert dusts used as fillers for insecticidal dusts, observations on china clay dusts have shown that with particles below 5 microns considerable aggregation occurs. Under field conditions it is very unlikely that bees would ever be exposed for prolonged periods to dust clouds of equivalent density to those generated in the above experiments.

It may, therefore, be anticipated that the entry of dusts into the respiratory system of the honey bee is not likely to be a major factor affecting toxicity when insecticidal dusts come into contact with the honey bee in the field.

Summary.

A description is given of the hair structures associated with the spiracles of the adult worker honey bee, *Apis mellifera* L.

The surface hairs around the spiracular orifices vary in size, density and arrangement and with the exception of those of the third, fifth and sixth abdominal spiracles appear to be capable of holding back particles greater than 30 microns.

When living bees were exposed to dust clouds of charcoal and cuprous cyanide, no particles were found in any internal part of the respiratory system beyond the spiracles except in the case of the trachea of the first thoracic spiracle which contained charcoal particles less than 5 microns.

The mechanism of tracheal ventilation was considered and evidence accumulated to suggest that all spiracles could have an inspiratory function.

Acknowledgements.

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References.

- FRAENKEL, G. (1932). Untersuchungen über die Koordination von Reflexen und automatisch-nervösen Rhythmen bei Insekten. III.—Z. vergl. Physiol., **16**, pp. 418–443.
- HAMILTON, A. G. (1937). The mechanism of respiration of locusts and its bearing on the problem of inhalation of poison dusts.—Bull. ent. Res., **28**, pp. 53–68.
- SNODGRASS, R. E. (1925). Anatomy and physiology of the honeybee.—327 pp. New York, McGraw-Hill.
- WEBB, J. E. (1945a). The penetration of derris through the spiracles and cuticle of *Melophagus ovinus*, L.—Bull. ent. Res., **36**, pp. 15–22.
- WEBB, J. E. (1945b). On the respiratory mechanism of *Melophagus ovinus* L. (Dipt.).—Proc. zool. Soc. Lond., **115**, pp. 218–250.
- WOHLGEMUTH, O. E. (1929). Die Atemmale (Stigmen) der Honigbiene.—Erlanger Jb. Bienenk., **7**, pp. 1–46.
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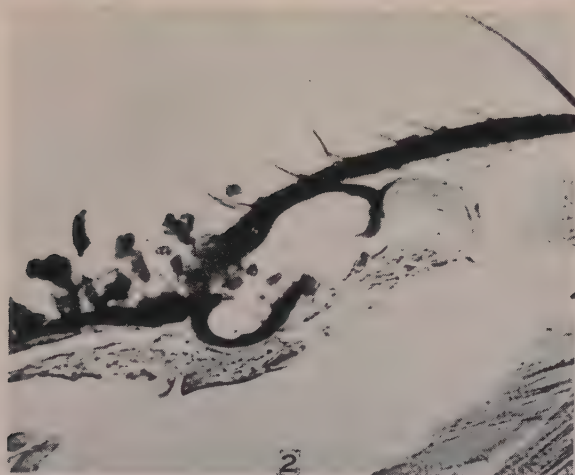
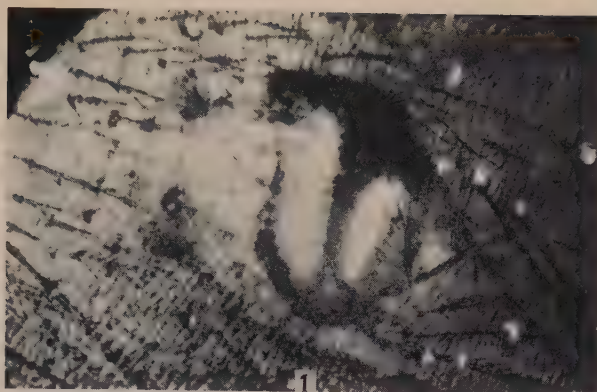
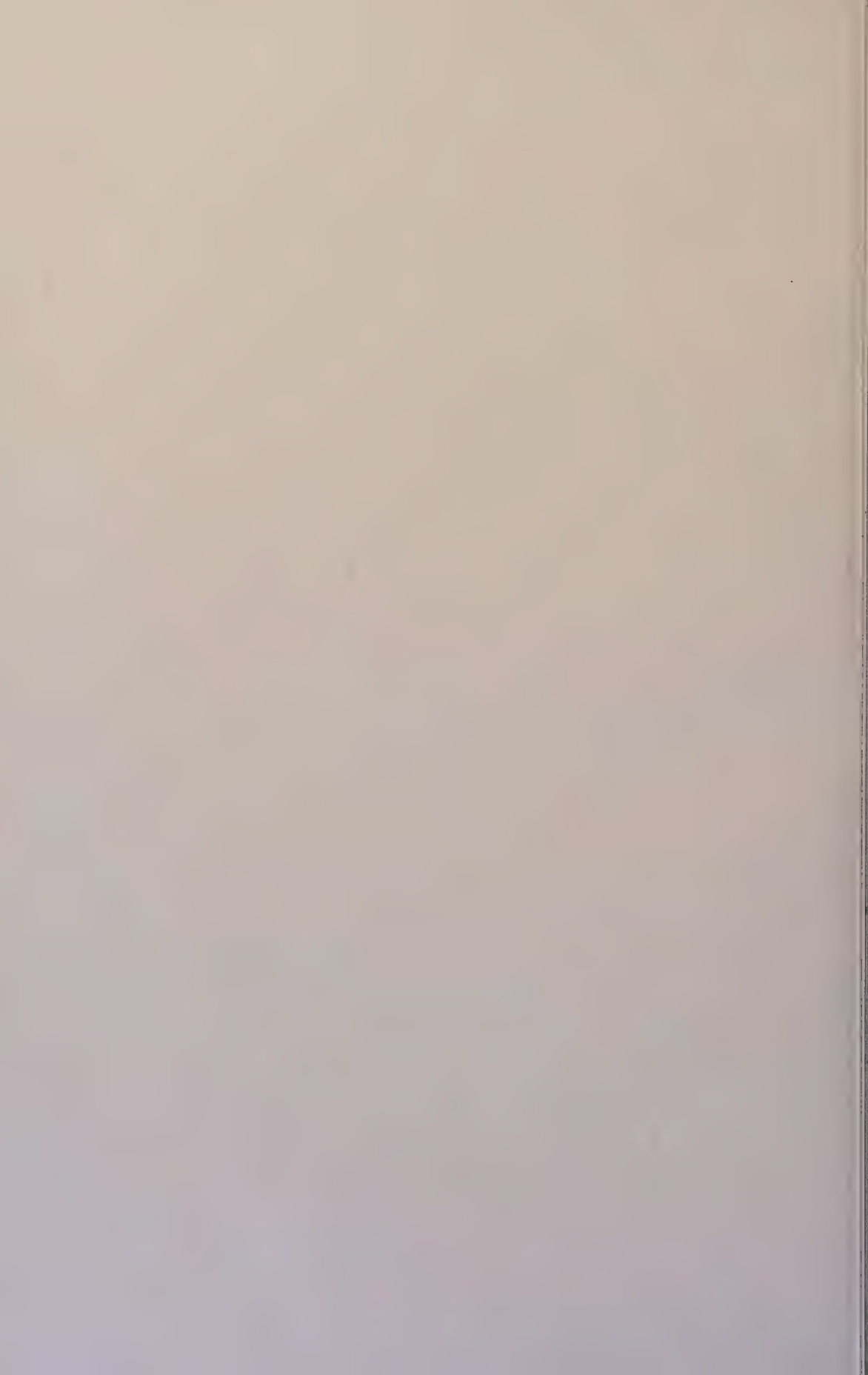


FIG. 1. Surface view of third abdominal spiracle. (X 174.)
FIGS. 2 and 3. Transverse sections through abdominal spiracles, showing dust particles trapped within spiracular atrium. (X 174.)



FORMATION OF GREEN PIGMENT AND COLOUR CHANGES IN ORTHOPTERA.

By Salâhattin OKAY.

Department of Zoology, University of Ankara.

The factors which lead to the production of green pigment in dichroic Orthoptera have been investigated with divergent results. Some authors claim that the deposition of green pigment, like that of grey, orange-yellow and reddish-brown is determined by the background (Chauvin, 1941; James, 1944; Roonwal, 1947; Ergene, 1950). Others have found that a green background is without effect on the production of green pigment (Przibram, 1907; Faure, 1932; Hertz & Imms, 1937). Faure obtained green individuals only when they were fed on growing grass in a humid atmosphere.

The first experiments in this paper were carried out on *Mantis religiosa* L. and *Acrida* sp. in the University of Ankara during 1949 and 1950. The investigation was continued with *Locusta migratoria migratorioides* (R. & F.), *Schistocerca gregaria* (Forsk.), *Dixippus morosus* Brunn. and again *M. religiosa* in the University of Cambridge during 1951.

I. BACKGROUND AND FEEDING EXPERIMENTS.

Wooden cages, 20×20×20 cm. were used for background experiments. Green-, brown- or yellow-coloured netting was placed around and on the top of the cages; the bottom was covered with a crepe paper of the same colour. All the experiments were carried out indoors.

A. *Mantis religiosa*.

This species has been investigated previously by two workers. According to Przibram (1907), the newly hatched nymphs are brown. They may become green later; but this is not dependent upon the green background. Most of the nymphs kept in cages become more or less yellow in the adult stage irrespective of the colour of the cage. James (1944) claims that *Mantis* nymphs can adapt their colour in some cases to the green or brown background, but recognises that his results are not conclusive. Indeed he carried out no control experiment.

During the summer of 1949, 115 nymphs and adults were collected in the field. Of the 43 males, 30 were green and 13 brown whilst of the 72 females, 56 were green, 13 brown and 3 yellow. Green and brown individuals were often found side by side and on the same substrate. The experiments were begun on 6th August, 1949, and carried on until the immature specimens reached the adult stage. The nymphs were mostly fourth and fifth instar, the normal number of instars being seven in this species.

They were often kept in separate cages, but sometimes together. *Calliphora* were given to the fourth-instar nymphs as food and *Oedipoda* to older nymphs. Very few nymphs died during the experiments.

(a) *Green nymphs on brown background.*

Nymphs tested	28
„ becoming brown	12+5 (more or less brown).
„ remaining green	11
Percentage becoming brown	60.7 per cent.

More than half of the green nymphs became brown on this background. But this is not an adaptation as will be seen from the next experiment.

The colour change takes place after moulting. The nymphs become olive-green temporarily, as described by James, then gradually brown. The colour change is very slow, and takes 4-5 days. Out of the 17 which became brown, nine did so at the last ecdysis.

(b) *Green nymphs on green background.*

Nymphs tested	21
„ becoming brown	10+2 (more or less brown)
„ remaining green	9
Percentage becoming brown	57 per cent.

More than half of the green nymphs again became brown on the green background. These two experiments show that the nymphs have a tendency to lose their green pigment irrespective of the background.

(c) *Brown nymphs.*

The brown nymphs are always fewer than the green in nature. Only 11 specimens were available for this experiment. Six of them were put in green cages and the remaining five in brown. None of them became green.

(d) *Laboratory-reared nymphs on white background.*

The author observed the development of 17 *Mantis* from hatching until the adult instar during 1951. Several nymphs were kept together in wooden cages covered with white netting. The newly hatched nymphs have a brown integument. They were fed with *Drosophila* in the very young stages, then mostly with *Calliphora*.

All the nymphs without exception became green in the early instars. The first brown nymph appeared in the fifth, the second one in the following instar; two in the pre-adult and one in the adult instar. The others remained green throughout.

These observations in white cages show that the formation of green pigment in young nymphs has nothing to do with a green background. These observations indicate that in the previous cage experiments (a) those of the green nymphs which became brown during the older stages of development may have done so irrespective of the brown background. Many of the green nymphs in the cage experiments carried out by James may likewise have become brown at the pre-adult and adult instars. This colour change is not due to the background, as that author believed, but is certainly connected with the metabolism of the older nymphs and adults. None of the sixth-instar brown nymphs in the present work became green in the green cage experiments. According to James, one brown pre-adult instar became green, but other older nymphs did not.

The present observations and those of James show that the young nymphs can readily produce the green pigment, but a number of the older nymphs lose this capacity.

The rearing experiments from hatching up to the adult stage having indicated that the appearance of brown pigment is not a background effect, an explanation must be sought elsewhere. It is suggested that in nature its appearance in some young nymphs may be due to insufficient nourishment. These nymphs cannot easily become green later. This might perhaps be tested by feeding the nymphs from the first instar on different species and different quantities of insects.

B. *Acrida* sp.

This is a large and sluggish Acridid. It is rather common around Ankara.

Vosseler (1902) reports that the green individuals of *Truxalis* (*Acrida*) *unguiculata* Ramb. live on the fresh grass of the oases and the yellow ones on stubble-fields. According to the observations of Haviland (1926) in June, while the grass is still fresh and green, *Acrida* sp. is green. In August, when the herbage is dried and yellow, the grasshoppers still feed in the same spots, but now they are brown and scorched-looking in the perfect similitude of bits of straw. According to Ergene (1950), the majority of the *Acrida* nymphs are green in a green field, brown and yellow in a dry yellow one. The numbers of green and yellow nymphs were nearly equal in another field where there was a mixture of green and dry grass.

Observations in nature made during the present work agree with those of previous authors. For example an observation, similar to that of Haviland, was made in a field of wheat. As this suggested that this colour change is determined by the background, the summer of 1949 was spent in experimenting with coloured cages.

Background.

The nymphs were kept in cages similar to those used for *Mantis*. It is rather difficult to rear *Acrida* in cages and the mortality may sometimes be very high. The nymphs hatch towards the middle of June around Ankara. The experiments began on 2nd July, 1949, with material collected in the field. The first nymphs found were probably second instar. The nymphs which underwent one moult in the cages are incorporated in the list given below; the survivors were followed through up to the adult instar. The grass, mostly *Triticum repens*, was changed every other day.

(1) *Green nymphs in yellow cages.*

Nymphs tested	33
„ becoming yellow (or grey)	15
„ remaining green	18
Percentage becoming yellow	45.4

The colour change occurs generally after a moult, but it was observed that a few young nymphs lost their green pigment before ecdysis. The colour change after moulting is quick in young nymphs which look very pale yellow during ecdysis, and become yellow or grey within 2-3 hours. The colour change is much slower in old nymphs. A few young green nymphs, which became yellow without moulting, looked as if they were gradually losing their green pigment which disappeared entirely within two days.

(2) *Green nymphs in green cages.*

Nymphs tested	40
„ becoming yellow (or grey)	21
„ remaining green	19
percentage becoming yellow	52.5

These results are very similar to those obtained in yellow cages. One may conclude that half of the green nymphs lose their green pigment irrespective of the colour of the cages, when they are fed with grass changed every other day.

(3) *Yellow or grey nymphs in green cages.*

Nymphs tested	46
„ becoming green	5
„ remaining yellow	41
Percentage becoming green	10.9

Only five yellow nymphs became green in green cages, whereas 41 remained unchanged. This colour change, confirmed the following year on fresh growing grass, always occurs after an ecdysis. It happens very quickly; the integument appears pale green during moulting, then it becomes entirely green. Two out of these five remained pale green.

(4) *Yellow or grey nymphs in yellow cages.*

Nymphs tested	45
„ becoming green	2
„ remaining yellow	43
Percentage becoming green	4.4

One became green and a second became only pale green, in yellow cages. These results are more or less similar to those obtained in green cages. The great majority of the yellow or grey nymphs remain as they are irrespective of the colour of the cages when they are fed with grass changed every other day.

The following conclusions may therefore be drawn: a green background does not help to maintain the green pigment in the nymphs; and a green background cannot be the cause of the formation of green pigment in the integument.

Dry and growing green grass.

During the summer of the following year, the effect on *Acrida* of dry and growing green grass as food was investigated. All the experiments were performed out of doors. The cages were exactly the same as in the earlier experiments.

(1) *Dry grass* (the grass was changed every 4–5 days).

(a) *Green nymphs in green cages.*

Nymphs tested	48
„ becoming yellow (or grey)	37
„ remaining green	11
Percentage becoming yellow	77.1

(b) *Yellow nymphs in green cages.*

Nymphs tested	37
„ becoming green	0
„ remaining yellow	37
Percentage becoming green	nil

These experiments show that the majority of the nymphs (77.1 per cent.) lose their green pigment if they are fed on dry grass; nor can this pigment be formed in the integument when the yellow or grey nymphs are fed on this grass.

It is interesting to note that the green or yellow *Acrida* excrete pink faeces when they are fed on dry grass. This is a redox brown pigment. According to Chauvin (1941) *Schistocerca* nymphs and adults excrete the same pigment, especially if they are fed on bran. Goodwin & Srisukh (1950) proposed the name insectorubin for this pigment in *Locusta* and *Schistocerca*.

(2) *Growing green grass.*

Faure (1932) obtained a high percentage of green *Locusta* and *Locustana* on growing grass. In the present experiments the cages were covered with white netting and were put on growing grass, which was mostly *Triticum repens*. The position of the cages was changed from time to time, when the grass was no longer fresh.

(a) Green nymphs.

Nymphs tested	50
„ becoming yellow	11
„ remaining green	39
Percentage becoming yellow	22

It had been hoped that all the nymphs would remain green under these conditions, but only 78 per cent. did so. Nevertheless, this percentage is much higher than in the previous experiments, as shown below.

Food	Percentage remaining green
Dry grass	22.9
Grass changed every other day	51
Growing green grass	78

(b) Yellow or grey nymphs.

Nymphs tested	43
„ becoming green	30
„ remaining yellow	13
Percentage becoming green	69.8

The percentage which became green is much higher here than in the previous experiments, as shown below.

Food	Percentage becoming green
Dry grass	0
Grass changed every other day	10.9
Growing green grass	69.8

The majority of the nymphs and adults were brilliant green; only four were pale green.

These results and those of the previous year do not confirm the observations of Ergene (1950), who investigated the same species. This author reared *Acrida* nymphs differently on green and yellow backgrounds. The nymphs, which were on a green background, received green grass changed every day: "Bei diesen Versuchen wurden die Käfige mit grünen Gras ausgelegt" (p. 536). The others, which were on a yellow background, received merely yellow grass or straw. "Wenn der Käfig als gelben Untergrund Getreidehalme enthielt, wurde kein anderes Futter hinzugefügt" (p. 536) and "... dass grüne Larven auf gelben Teilen von panaschierten Blättern genau so gelb wie grüne Larven auf gelben Gras oder trockenen Getreidehalmen wurden" (p. 546).

Ergene claims that *Acrida* has a colour adaptation to background, which is not affected by the colour of food (p. 546). The error of this conclusion would have been apparent to her if she had tried to rear both the yellow and green nymphs with yellow grass or straw in a green cage. Her paper does not seem to prove an adaptation to background, but merely to support the view expressed here.

The present work supports the findings of Faure on *Locusta* and *Locustana*. This author obtained, with both species, a higher percentage of green insects than in *Acrida* by rearing on growing green grass under celluloid boxes.

Species				Percentage of green
<i>Locusta migratoria</i>	*	(Faure)	...	86.3
<i>Locustana pardalina</i>	...	(Faure)	...	72.9
<i>Acrida</i> sp.	...	(Okay)	...	69.8

Observations on the Haemolymph.

The green pigment in the integument and haemolymph of the Orthoptera and Lepidoptera is known to be a combination of a yellow carotene-protein with a blue bile pigment-protein (Junge, 1941; Okay, 1945, 1947; Goodwin & Srisukh, 1951). According to these authors, the blood contains the same pigments when it is green, but the yellow component (carotene-protein) is also often present in the integument and blood of non-green varieties. The appearance of green colour depends, therefore, on the formation of the blue bile pigment-protein.

Firstly, it would be interesting to know how often the green varieties possess a green blood and, secondly, how often the colour of the blood alters after a colour change.

The colour of the blood was examined and compared on pieces of filter paper. The nymphs were pricked at the base of the hind leg with a fine needle and the drops of blood were left to dry on filter paper and then compared. The colour was very distinct at the border of the drops. It is possible to test the blood of a nymph several times with a very fine needle, without damaging it.

The blood of 182 green nymphs collected from grassy areas was examined and 164 (90 per cent.) were found to possess green blood. On the other hand, not one of 123 yellow or grey nymphs collected from dry grassy areas had green blood. These observations show that there is a close connection between the green pigment of the integument and in the blood.

During the feeding experiments on yellow nymphs with growing grass, the haemolymph was examined every three days. The blood is yellow or sometimes colourless in yellow nymphs. The blood becomes gradually green, but often the change seems very rapid. Eleven out of 17 nymphs had a green blood before they became green. Six others had green blood only 2-3 days after they had become green.

These observations show a close relation between the green or, more correctly, the blue bile pigment of the blood and integument which often appeared in the blood before it was deposited in the integument.

These investigations were continued during 1951 in the University of Cambridge. The purpose was to try to find out the mechanism of the formation and disappearance of the green or blue bile pigment, and eventually its precursor.

C. *Locusta migratoria migratorioides* and *Schistocerca gregaria*.

The experiments were carried out mostly with *Locusta*. Some were repeated with *Schistocerca* but no essential difference between the two species was found. All the experiments were done in the laboratory.

Green background.

There is no need to stress this point. *Locusta*, like *Acrida*, does not adapt itself to the green background. Newly hatched nymphs are never green. None of them became green at any stage when they were kept separately in small green cages with grass changed every two days.

Some of the nymphs had more or less green blood in the second and third instars. But this pigment disappeared later and was never seen in the integument.

Growing grass.

The nymphs were mostly fed on wheat growing in boxes, but sometimes on Yorkshire fog (*Holcus lanatus*). The boxes of wheat were changed when the foliage began to lose its freshness. It was noticed, from preliminary experiments, that *Locusta* nymphs may become green, even though they are kept together in small numbers. Ten to twelve nymphs were always put in the same cage, but this did not prevent the formation of green solitary colour. No discrimination was made between solitary and gregarious hatchlings.

The nymphs were first reared in $50 \times 40 \times 50$ cm. wire cages, heated with a 60-watt bulb. Some died during the first instar. The nymphs became green generally between the second and fourth instars. A total of 146 nymphs was tested of which 61 (42 per cent.) became green. The colour change occurs quickly after moulting, the solitary nymphs becoming green more readily than the gregarious ones. But nymphs usually became more or less pale before the moult which precedes the colour change.

Later the nymphs were reared in 20×35 cm. celluloid cylinders, heated with 60-watt bulbs. The great majority of the nymphs, 98 out of 121 or 81 per cent., became green under these conditions. This percentage is nearly double that obtained in wire cages. Although both the cages and cylinders were heated by bulbs of the same power, the temperature was evidently higher in the latter. The humidity was also higher in the cylinders because of the reduced amount of ventilation but it cannot be kept up in wire cages. Faure (1932) believed that a humid atmosphere is necessary for the formation of green pigment. The effect of temperature and humidity was not examined accurately, but the following experiments were performed.

The cylinder was heated with two 60-watt bulbs and the nymphs fed with cut grass changed every three days. None of the nymphs became green, but all were pale. According to Husain and Ahmad (1936), locusts reared at high temperature are pale and at low temperature dark. Some of the nymphs in the present experiment had a slightly green blood, but this pigment never appeared in the integument.

In a second series of experiments, the cylinder was heated with a 60-watt bulb. The bottom of the cylinder was filled with water; wet cloths were added inside it to keep the atmosphere moist. The nymphs were fed with cut wheat changed every three days. The result was the same; none of the nymphs became green but they were less pale than in the previous experiment.

These experiments suggest that neither temperature nor humidity can induce the formation of green pigment without fresh grass. But the comparison of the percentages of green nymphs obtained in cages and cylinders suggests that these factors are not without effect on the formation of green pigment.

Those nymphs which become brilliant green on growing grass always have green blood. The blood of the dark green forms may not have this colour.

The formation of green pigment in the integument and blood is reversible at any time. Green nymphs, which are transferred from fresh grass to dry grass, lose their green pigment after a certain time and their blood becomes yellow or colourless. The disappearance of green pigment is gradual and it may sometimes be complete before the following moult.

The loss of green pigment occurs also in immature adults, even in a solitary cage, when they are transferred to dry grass. This phenomenon was not observed in *Acrida*. When brown adults or dark nymphs with non-green blood are fed on growing grass, the green pigment may appear in their blood.

These observations show clearly that the formation of green pigment depends very largely on the food.*

Schistocerca gregaria.—Feeding experiments have been repeated using this species. There is a difference between newly hatched solitary nymphs of *Locusta* and *Schistocerca*. The latter are grey green. The blood of newly hatched solitary and gregarious nymphs is almost always green. This species generally forms green pigment more easily than *Locusta* and keeps it longer.

Similar results were obtained by feeding *Schistocerca* on growing wheat and dry grass. The solitary nymphs kept on fresh wheat remain green during the nymphal stages; the gregarious ones become green in the second instar and keep that colour until the adult instar. Their blood is always brilliant green.

Nymphs fed on cut wheat changed every three days never form green pigment either in the integument or in the blood. The formation of green pigment is reversible at any stage, as in *Locusta*. The green nymphs transferred from growing grass to dry grass lose this pigment in the integument and blood after a certain time.

Chauvin's observations (1941) on this species have not been confirmed. The coloured cage experiments of this author are not easy to understand. "Les solitaires tendent, toujours, plus ou moins à prendre une teinte verte, plus fréquemment si le fond est vert, mais aussi s'il est noir, blanc ou jaune" (p. 239). But he claims, on the following page, that a green background is quite sufficient for the production of green pigment and that green growing grass is absolutely unnecessary. If the grass or lettuce is changed every day, as is mentioned in the paper, it would be sufficient to produce green pigment in this species, for it has already been pointed out that *Schistocerca* can form green pigment more easily than *Locusta* and keeps it longer. The formation of green individuals on black, white and yellow backgrounds, as was obtained by this author, may be explained in the light of the present work.

Darkness.

Some rearing experiments were carried out in darkness to eliminate the effect of the green background of growing grass. A celluloid cylinder with its growing wheat was placed in an incubator at 29°C. The foliage soon became yellow in darkness, so the box of wheat had to be changed every three or four days. There is a second difficulty; the air in the incubator is quickly saturated with moisture from the transpiration of the wheat, which becomes almost wet. The mortality is very high. Of 112 *Locusta* nymphs only 14 survived of which eight became green at the second, third and fourth instars. Five of the green nymphs were quite similar to those obtained in light; three were dark green.

The mortality is much less on dry grass. Reared on this food, the nymphs became blackish, buff or brown.

A similar experiment was made by Poulton (1894) on *Tryphaena* (Lepidoptera) larvae. These larvae were kept in darkness, from hatching, on cabbage leaves. The author did not ensure the freshness of the leaves; some of the larvae became green, but some brown.

According to MacBride and Jackson (1915), the nymphs of *Dixippus*, which are kept, from hatching, in complete darkness, may grow up as pure green individuals.

These experiments show that the green pigment may be formed in darkness.

*The formation of green pigment in the blood is not confined to dichroic grasshoppers. The author had the opportunity of observing green blood in some *Oedipoda miniata* (Pall.), which is not a dichroic Orthopteran. There is no doubt that this pigment in the haemolymph is connected with the food. It is quite possible that the same pigment exists also in the integument of *Oedipoda*, but it cannot be seen because of the dark cuticle.

D. *Dixippus morosus*.

This species has already been investigated, among other authors, by Toumanoff (1927, 1928) and Giersberg (1928). Experiments described below confirm their results. The newly hatched nymphs are brown but, when they are fed on fresh privet, they become green within a week while still in the first instar.

Nymphs fed on dry privet in a humid atmosphere become brown. It is noteworthy that nymphs and adults, which are fed on dry privet leaves, never excrete pink faeces.

Nymphs reared on red radish and white potato become greenish-blue in the second instar. These specimens certainly contain very small quantities of carotene-protein, the yellow component of green pigment. The formation of the blue bile pigment on a non-green background or in darkness shows that the production of this pigment is not related to a green background.

The blood of *Dixippus* is different from that of the species studied hitherto, since the blue pigment in the haemolymph does not disappear in brown or grey varieties.

Conclusions.

Green and brown (or yellow) backgrounds have no effect on the formation of green (blue bile pigment—protein carotene-protein) and brown pigments in species of Orthoptera studied in this paper. The yellow component has a limited effect on the coloration of the integument. The colour changes from brown (or yellow) to green, or *vice versa*, are entirely based on the formation or disappearance of the blue pigment. The brown pigment may exist in large amount in the integument of some green grasshoppers (*Locusta*, *Acrida*) under the blue pigment. The formation of blue pigment depends mostly on the freshness of the grass on which the insects are fed. Temperature and humidity may have some effect on the formation of this pigment. Like *Dixippus* (MacBride & Jackson, 1915), *Locusta* may become green in complete darkness.

When brown or yellow nymphs are transferred from a poor food to fresh grass, the blue pigment appears generally first in the blood. This pigment will increase after a certain time and be deposited in the integument at the following moult. As the blue pigment lies on the brown and any other pigment which may be present in the integument, these latter will be concealed.

On the other hand, when green nymphs are transferred from fresh grass to more or less dry grass, no further blue pigment can be synthesised and deposited in the integument. The blue pigment already deposited will disappear generally after, but sometimes before an ecdysis (*Acrida*). The blue pigment disappears also from the blood.

There are several reasons for believing that the colour change occurs in the same way in carnivorous Orthoptera. *Mantis* shows no colour adaptation to green and brown backgrounds. The blue pigment is a bile pigment and it exists in the blood of green varieties (Okay, 1947). The brown pigment is similar to insectorubin (Okay, 1948). Generally all the young nymphs are green. Some of the old nymphs and adults have a tendency to lose their blue pigment and to become brown under the same conditions of background (white) and food supply (*Calliphora*). This colour change may be due to a modification in the metabolic activity of some old nymphs and adults.

It is concluded that the colour change in Orthoptera from green to non-green, or *vice versa*, is not influenced by background, but offers an example of nutritional homochromy. It is quite possible that the non-green individuals may have a limited

adaptation to background as has already been recorded by Faure (1932) and Hertz and Imms (1937).

II. EXPERIMENTS ON FORMATION OF GREEN PIGMENT.

An attempt will be made, in this section, to explain the mechanism of the appearance and disappearance of the green, or more correctly, the blue bile pigment in the integument and blood.

Administration of Chlorophyll.

For these experiments water-soluble chlorophyll which is prepared by British Drug Houses Ltd., was used. It was administered by mixing it with the food of two phytophagous species, *Locusta* and *Schistocerca* and by injecting it into their bodies.

The experiments already described in Part I show that if the nymphs are fed on dry grass, the blue pigment appears neither in the integument nor in the blood. Likewise it was found that *Locusta* and *Schistocerca* cannot form this pigment when fed on bran or bread. Growth is better on bread ; but very few can reach the adult instar. The green pigment never appeared either in the integument or in the blood which remained colourless like water.

Feeding experiments.

Pieces of bread were mixed with a fairly concentrated solution of chlorophyll. The food was changed every day. *Locusta* nymphs, immediately after hatching, were reared on this medium in celluloid cylinders. The nymphs did not feed with eagerness, and growth did not go beyond the fourth instar ; the integument never appeared green. The blood, which was colourless at birth in this species, was checked from time to time. It remained colourless during the experiments. The chlorophyll was therefore apparently not absorbed as chlorophyll nor, if it was absorbed at all, was it converted into the blue bile pigment as it crossed the gut wall.

Solitary *Schistocerca* nymphs were fed on bread+chlorophyll. The blood is green at birth in this species, at least in solitary forms. Newly hatched nymphs, which were put on this food, lost their green pigment in the blood at the second instar. The haemolymph remained colourless during the rest of the experiments. Many nymphs died in the third and fourth instars. There was no sign of absorption of chlorophyll.

Injection experiments.

A chlorophyll solution in physiological saline was injected into third, fourth, and fifth instars of non-green *Locusta* with yellow or colourless blood. The nymphs were kept on dry grass after injection to prevent the natural formation of blue bile pigment and its appearance in the blood.

The nymphs cannot support a concentrated solution of chlorophyll, and soon die. A diluted solution was therefore used and those which looked healthy after the injection, received a second or a third after an interval of two or three days. No trace of green pigment appeared in the integument after moulting. The blood was green because of the presence of chlorophyll. This colour might be seen 17 days after the injection.

The pericardial cells were examined after the injection. It is known that these cells may absorb colloidal particles from the blood. But since these cells are often blue in fourth and fifth instars of green and non-green *Locusta*, this species is not suitable for such an observation.

On the other hand *Schistocerca* nymphs often have a colourless heart : the blue pigment is sometimes present, but in small quantities. In experiments with this

species, which was kept on dry grass, small quantities of chlorophyll were found to be absorbed by the pericardial cells. It seems that the anterior part of the heart absorbs more pigment than the posterior part. The chlorophyll may be seen in the pericardial cells 15 days after the injection, but no green pigment is seen in the integument.

It may be recalled here that *Dixippus*, which is also a phytophagous Orthopteran, may form blue bile pigment in the absence of chlorophyll, as was shown in rearing experiments on red radish and white potato (p. 307).

Administration of Haemoglobin.

All plants possess haematin in very small quantities. The breakdown of this pigment into the blue bile pigment is a possibility.

Pieces of bread were mixed with bullock's blood. Many of the *Locusta* and *Schistocerca* nymphs died on this food, which was changed twice daily. Some of the nymphs reached the third instar, but the haemoglobin was not absorbed.

Diluted laked blood was injected into the haemolymph of the third-fifth instars of non-green *Locusta*, which were fed on dry grass or bread. A blue or green pigment did not appear in the blood, which was generally reddish or orange. The pericardial cells are blue in the nymphs of this species and therefore they are not suitable for examination for the presence of absorbed haemoglobin or its derivatives.

The pericardial cells of *Schistocerca* absorb very small quantities of haemoglobin. The pigment appears reddish-brown and is in a compact form. No conversion into a green or blue pigment was observed, even 11 days after the injection.

As already mentioned (see p. 300) the young nymphs of *Mantis* fed on *Calliphora* are green. It is known that this Dipteran contains a relatively large amount of cytochrome c in its muscles. A concentrated solution of cytochrome c of the horse was injected into the fifth-sixth instars of brown *Mantis* which possess a slightly brown blood. The injection was repeated in some of these nymphs. No blue pigment was observed in the blood.

Spectroscopic Study.

Some spectroscopic studies were carried out to investigate the presence of haematin in green *Locusta*, and in the eggs of *Dixippus*.

The gut was removed before extraction which was made in pyridine + $\text{Na}_2\text{S}_2\text{O}_4$ in Thunberg vacuum tubes. The solution is brownish because of the presence of insectorubin (Goodwin & Srisukh, 1950). Some extractions were made after the removal of carotenoids with ether. Green and non-green nymphs were compared. Both possessed only a faint band of cytochrome c at $550 \text{ m}\mu$ and there was no difference between the two varieties. The haematin band was absent.

The first-instar nymphs of *Dixippus*, unfed, have a green or blue blood two days after hatching. There is no doubt that the precursor of the blue pigment exists in the eggs, as in *Schistocerca*. Two hundred eggs, nearly ready to hatch, were crushed and extracted in Thunberg tubes. The pyridine solution was orange-yellow because of the carotenoids. It showed a faint band at $630 \text{ m}\mu$. The haematin band was absent.

The Pigments in the Fat Body and Pericardial Cells.

Pigments in the fat body.

At the beginning of the present experiments it was thought that the blue pigment appears first in the blood and is then deposited in the integument of *Acrida* (Okay,

1951). A closer investigation, however, suggested that the blue pigment might be formed directly in the fat body.

The fat body in insects is generally separated into central and peripheral layers. These two parts can easily be distinguished in *Locusta* and *Schistocerca*: (1) A yellow-orange central fat body, sometimes with a greenish tinge, which is much more voluminous than the peripheral layer. Seen under the microscope the yellow pigment is contained in fatty globules. All attempts to isolate the blue bile pigment from the central fat body have been unsuccessful. It yields only an epiphasic carotenoid which is a β -carotene (Goodwin & Srisukh, 1949). (2) The peripheral fat body, which is immediately under the skin, looks different from the central one. It is generally white or creamy white, in non-green nymphs of *Locusta* with yellow blood, and it contains a very small quantity of carotenoids. In grassy green nymphs, which were obtained in celluloid cylinders, the peripheral fat body is blue, especially in the anterior part of the body. The blue colour is not due to the underlying green pigment in the integument, because isolated pieces of fat body contain this blue pigment. Seen under the microscope, the blue pigment seems to be contained in fatty globules resembling the yellow globules of the central fat body. But they are smaller than the yellow carotenoid globules.

The peripheral fat body of brilliant green *Schistocerca* nymphs is also blue: the pigment is soluble in ether, ethyl acetate and chloroform, but not in water. It is judged by these properties that this pigment is not a chromoprotein, but a prosthetic group. It was only possible to try the Gmelin reaction on isolated tissues, because of the small quantities of the peripheral fat body: violet, orange and yellow colours were visible.

The blue pigment exists also in the peripheral fat body of green *Mantis* nymphs and adults. This species has generally an almost colourless (white) central fat body, with very little carotenoid. The blue pigment seems to be contained in fatty globules, as in *Locusta* and *Schistocerca*.

It was not possible to distinguish a central and a peripheral fat body in green *Dixippus*. The tissue is generally creamy white containing very little carotenoid in the abdomen. There are some very loose and pale masses between the fat body and integument.

Pigment in pericardial cells.

The pericardial cells of insects are known to contain sometimes yellow, brown, red or green pigments (Hollande, 1922).

The pericardial cells of many *Locusta* in the third-fifth instars are filled with a blue pigment. These cells are almost always blue in green nymphs of this species. They are sometimes blue, sometimes colourless in non-green nymphs with green blood and they are rarely blue in those with pale yellow or colourless blood. This pigment can be easily extracted from isolated pericardium by means of water. Gmelin's reaction can be seen clearly on pericardial cells.

The accumulation of the pigment in the cells is certainly attained gradually during growth, because the old nymphs possess more pigment in the pericardial cells than the young ones. The pigment, once deposited, is probably not returned to the blood, because it remains in the pericardial cells of starved fifth and immature adult instars, although their blood becomes pale yellow or even colourless after 10 days of starvation.

It is interesting to note that the yellow carotene-protein of the blood is probably not deposited in the pericardial cells of *Locusta*, because these cells always look blue, and the water extract of the pigment from isolated pericardium has a blue colour without a greenish tinge. The small quantity of the pigment make impossible a chemical detection of the carotene-protein.

A second pigment begins to appear in the pericardial cells of the fourth instar. It is a reddish-brown pigment. Both the blue and reddish-brown pigments can coexist in the fourth, fifth and sometimes in immature adult instars. The distribution and respective quantities of both pigments on the cells of different instars vary considerably. After the adult emergence, the pericardial cells generally become reddish-brown, although in some individuals they may still remain blue. But in mature adults, male or female, the pericardial cells are always reddish-brown, and never blue. The blue pigment, which is present during the nymphal stages is no longer visible in isolated pericardial cells under the microscope. These hearts never yield a blue chromoprotein in water, and Gmelin's reaction is never observed, *i.e.*, no bile pigment is now present. The brown pigment is so abundant in the mature adults that it is not only deposited in the pericardial cells, but also sometimes on the

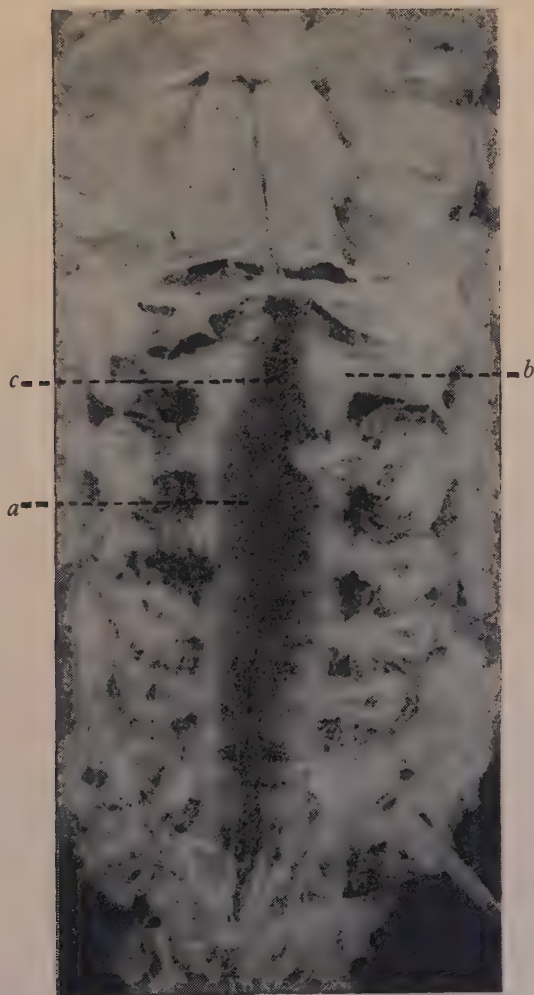


Fig. 1. Pericardium of a mature male *Locusta*. Dissection from ventral side with all other organs removed : (a) pericardial cells filled with insectorubin ; (b) peripheral fat body ; (c) heart.

fat body around the pericardium (fig. 1). The mature males possess more pigment than the females.

The pericardium of green nymphs of *Schistocerca* may sometimes be blue, although it is often colourless. The reddish-brown pigment often appears in the pericardium of the fifth instar, as has been observed by Chauvin (1941). The pericardium of mature adults, especially that of males, becomes full of brown pigment as in *Locusta*.

A blue pigment is always deposited in the pericardial cells of *Dixippus* nymphs and adults, but the pericardium is blue at every stage, including sexual maturity; a brown pigment is never observed in these cells.

Discussion of Results and Conclusions.

The experiments carried out in Part II may throw some light on the formation of green pigment in Orthoptera.

Metcalf (1945), who suggested that the phytophagous bug, *Anasa tristis* Deg., can break down chlorophyll into a derivative analogous to biliverdin, did not carry out feeding experiments. It was thought at the beginning of the present work that the blue pigment in *Acrida* might be a break-down product of chlorophyll (Okay, 1951). But this belief has not been confirmed by feeding experiments with nymphs of *Locusta* and *Schistocerca* since chlorophyll, as administered in these experiments was not absorbed by the gut. Moreover, it was shown in Part I of this work that *Dixippus* may develop its normal green pigment when no chlorophyll is present in the food. Also it is obvious that the blue pigment in carnivorous green Orthoptera (*Mantis*, etc.) cannot have a relation with chlorophyll.

Haemoglobin is not absorbed by the gut of *Locusta* or *Schistocerca*. Small quantities of injected haemoglobin are absorbed by the pericardial cells of *Schistocerca*, but no green or blue pigment appears in these cells. In this respect, the pericardial cells of these two species differ from those of blood-sucking insects, *Rhodnius* and *Pediculus*, which break down haematin into biliverdin (Wigglesworth, 1943).

Injected cytochrome c of the horse does not undergo a conversion into blue pigment in brown *Mantis*.

It can be accepted from these experiments that the blue pigment in Orthoptera cannot be considered as a breakdown product of chlorophyll or haematin. Moreover the haematin band is absent in green and non-green *Locusta* nymphs.

Possible site of formation of blue pigment.

The presence of a blue pigment with a positive Gmelin reaction in the peripheral fat body of grassy green nymphs of *Locusta*, *Schistocerca* and *Mantis* suggests the possibility that this pigment may be related to the blue bile pigment-protein in the blood.

A green pigment in the fat body of different green insects is reported by Poulton (1892), Buys (1924) and Metcalf (1945). Gmelin reaction was observed in the fat body of *Anasa* (Hemiptera) by the last author. Although not explicitly stated, the green pigment seems to have been only in the peripheral fat body. It seems probable that a blue pigment is mistakenly called green by these authors.

There are two possibilities to explain the presence of a blue pigment in the peripheral fat body of brilliant green nymphs: (a) The excess of blue pigment, which becomes abundant in the blood after ingestion of fresh grass, is stored in it. (b) The blue pigment is formed in this part of the body, then passes into the blood.

The fact that the blue pigment can only be seen distinctly in the peripheral fat body of brilliant green nymphs and that it cannot be seen in that of less brilliantly green individuals, whereas it is present in the blood, seems to favour the first possibility.

The blue pigment, however, is not present as a chromoprotein, but as a prosthetic group in the fat body, whereas the blue chromoprotein in the pericardial cells of *Locusta* nymphs and *Dixippus*, which it is thought may be absorbed from the blood, remains in them as a chromoprotein for some while or indefinitely. Moreover the negative results in the feeding and injection experiments mentioned above suggest that the blue pigment may be synthesised in the body. For these reasons it seems more probable that the blue pigment is synthesised from a colourless precursor and that this synthesis takes place in the peripheral fat body.

The possibility of conversion of blue pigment into insectorubin.

The green nymphs of *Acrida*, *Locusta* and *Schistocerca* kept on dry grass lose their green or, more exactly, blue pigment. This pigment is never excreted by the Malpighian tubes and is not present in the faeces. It must therefore be converted into another coloured or colourless substance.

Diazo reaction is negative on the blood of green and formerly green nymphs of *Locusta* and *Schistocerca*, indicating that the blue pigment has not been transformed into bilirubin.

The observation made on the pericardial cells of *Locusta* suggests that the blue pigment might be transformed into the reddish-brown one which, it is thought, may be insectorubin. Chemically such a conversion is not impossible. Blue pigment is considered as a bile pigment (Junge, 1941; Okay, 1945; Goodwin & Srisukh, 1951) and insectorubin has been identified as a N-methyl-pyrrole and haemopyrrole in *Locusta* and *Schistocerca* (Goodwin & Srisukh, 1950). Does this hypothetical conversion on the pericardial cells occur also in the integument? The slow colour change in *Mantis* gives this impression (p. 300). On the other hand, solitary green *Locusta* possess a large amount of insectorubin which, however, is masked by the green pigment (Goodwin & Srisukh, 1950). Likewise in green *Acrida*, the brown pigment is present, though concealed by the blue one, and can be seen from inside under the microscope (Okay, 1950). It is possible that the underlying insectorubin is a converted blue pigment, but the main objection to this conversion is certainly the case of the nymphs fed on dry grass. These can rapidly synthesise insectorubin, although no trace of blue pigment appears either in the blood or integument. To elucidate this question a histological study of the integument of different species of Orthoptera is being carried out in this laboratory.

Summary.

The colour change and formation of green pigment in *Mantis*, *Acrida*, *Locusta*, *Schistocerca* and *Dixippus* are studied.

There is no background reaction to green and brown or yellow colour in these species. Usually the young nymphs of *Mantis* are green; some of the old nymphs and adults have a tendency to lose the green pigment. Phytophagous nymphs become green only when fed on fresh or growing grass. Green individuals may be obtained in darkness on this food (*Locusta*). The green pigment disappears on a diet of dry grass.

The colour change from non-green to green, or *vice versa*, is dependent on the formation or disappearance of the blue component (bile pigment-protein) of green pigment. The blue pigment generally appears first in the blood and is deposited in the integument at the following moult; it does not appear to be a breakdown product of chlorophyll or haematin. It is probably synthesised from a colourless precursor in the peripheral fat body.

Observations made on the pericardial cells of *Locusta* suggest that the blue pigment may be converted into insectorubin.

Acknowledgements.

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References.

- BUSNEL, R. G. & DRILHON, A. (1942). Recherches sur la répartition de la riboflavine (vitamine B₂) et de quelques autres substances fluorescentes chez les insectes.—Arch. Zool. exp. gén., **82**, pp. 321–356.
- BUYS, K. S. (1924). Adipose tissue in insects.—J. Morph., **38**, pp. 485–528.
- CHAUVIN, R. (1938). Sur le rougissement du criquet pélerin.—C.R. Acad. Sci., **207**, pp. 1018–1020.
- CHAUVIN, R. (1941). Contribution à l'étude physiologique du criquet pélerin et du déterminisme des phénomènes grégaires.—Ann. Soc. ent. Fr., **110**, pp. 133–272.
- ERGENE, S. (1950). Untersuchungen über Farbanpassung und Farbwechsel bei *Acrida turrita*.—Z. vergl. Physiol., **32**, pp. 530–551.
- ERGENE, S. (1952). Farbanpassung entsprechend der jeweiligen Substratfärbung bei *Acrida turrita*.—Z. vergl. Physiol., **34**, pp. 69–74.
- FAURE, Jacobus C. (1932). The phases of locusts in South Africa.—Bull. ent. Res., **23**, pp. 293–405.
- GIERSBERG, H. (1928). Über den morphologischen und physiologischen Farbwechsel der Stabheuschrecke *Dixippus (Carausius) morosus*.—Z. vergl. Physiol., **7**, pp. 657–695.
- GOODWIN, T. W. & SRISUKH, S. (1949). The biochemistry of locusts. 1. The carotenoids of the integument of two locust species (*Locusta migratoria migratorioides* R. and F. and *Schistocerca gregaria* Forsk.).—Biochem. J., **45**, pp. 263–268.
- GOODWIN, T. W. & SRISUKH, S. (1950). Biochemistry of locusts. 3. Insectorubin : the redox pigment present in the integument and eyes of the Desert Locust (*Schistocerca gregaria* Forsk.), the African Migratory Locust (*Locusta migratoria migratorioides* R. & F.) and other insects.—Biochem. J., **47**, pp. 549–554.
- GOODWIN, T. W. & SRISUKH, S. (1951). Biochemistry of locusts. 5. The green pigment of the haemolymph and integument of solitary locusts (*Locusta migratoria migratorioides* R. and F. and *Schistocerca gregaria* Forsk.).—Biochem. J., **48**, pp. 199–203.
- HAVILAND, M. D. (Mrs. H. H. BRINDLEY). (1926). Forest, steppe and tundra. Cambridge.
- HERTZ, M. & IMMS, A. D. (1937). On the responses of the African Migratory Locust to different types of background.—Proc. roy. Soc., (B) **122**, pp. 281–297.
- HOLLANDE, A. C. (1922). La cellule péricardiale des insectes (cytologie, histochimie, rôle physiologique).—Arch. Anat. micr., **18**, pp. 85–307.
- HUSAIN, M. A. & AHMAD, T. (1936). Studies on *Schistocerca gregaria* Forsk. II. The biology of the Desert Locust, with special relation to temperature.—Indian J. agric. Sci., **6**, pp. 188–262.
- JAMES, H. G. (1944). Colour changes in *Mantis religiosa* L.—Canad. Ent., **76**, pp. 113–116.

- JUNGE, H. (1941). Über grüne Insectenfarbstoffe.—Hoppe-Seyl. Z. physiol. Chem., **268**, pp. 178–186.
- MACBRIDE, E. W. & JACKSON, A. (1915). The inheritance of colour in the Stick-insect, *Carausius morosus*.—Proc. roy. Soc., (B) **89**, pp. 109–118.
- METCALF, R. L. (1945). A study of the metabolism of chlorophyll in the Squash Bug *Anasa tristis* DeGeer.—Ann. ent. Soc. Amer., **38**, pp. 397–402.
- OKAY, S. (1945). Pigmentation of Orthoptera.—Nature, **155**, p. 635.
- OKAY, S. (1947). Contribution à l'étude du pigment vert chez les insectes.—Rev. Fac. Sci. Univ. Istanbul, (B) **12**, pp. 89–106.
- OKAY, S. (1948). Sur le pigment brun des Orthoptères.—Commun. Fac. Sci. Ankara, **1**, pp. 178–186.
- OKAY, S. (1951). Formation of green pigment in grasshoppers.—Nature, **168**, p. 254.
- POULTON, E. B. (1892). Further experiments upon the colour relation between certain Lepidopterous larvae, pupae, cocoons, and imagines and their surroundings.—Trans. ent. Soc. Lond., **1892**, pp. 293–487.
- POULTON, E. B. (1894). The experimental proof that the colours of certain Lepidopterous larvae are largely due to modified plant pigments derived from food.—Proc. roy. Soc., **54**, pp. 417–430.
- PRYOR, M. G. M. (1940). On the hardening of the cuticle of insects.—Proc. roy. Soc., (B) **128**, pp. 393–407.
- PRZIBRAM, H. (1907). Aufzucht, Farbwechsel und Regeneration unserer europäischen Gottesanbeterin (*Mantis religiosa* L.).—Arch. EntwMech. Org., **23**, pp. 600–614.
- ROONWAL, M. L. (1947). Studies in intraspecific variation. I.—Rec. Indian Mus., **44**, pp. 369–374.
- TOUMANOFF, K. (1927). Sur le rapport entre la formation du pigment vert figuré et la nutrition chez *Dixippus morosus* Br. et Redt.—C.R. Soc. Biol., **96**, pp. 1392–1393.
- TOUMANOFF, K. (1928). Le rapport entre la pigmentation et l'alimentation chez *Dixippus morosus*, Br. et Redt.—C.R. Soc. Biol., **98**, pp. 198–200.
- VOSSELER, J. (1902). Beiträge zur Faunistik und Biologie der Orthopteren Algeriens und Tunesiens. II. Theil.—Zool. Jb. (Syst.), **17**, pp. 1–99.
- WIGGLESWORTH, V. B. (1943). The fate of haemoglobin in *Rhodinus prolixus* (Hemiptera) and other blood-sucking arthropods.—Proc. roy. Soc., (B) **131**, pp. 313–339.

ON A BOSTRYCHID WOOD-BORER IN THE SUDAN.

By F. G. G. PEAKE, M.A., M.Sc.

Forest Entomologist, Anglo-Egyptian Sudan.

There is no doubt that the most outstanding wood-borer in the Sudan is the Bostrychid beetle, *Sinoxylon senegalense* Karsch, which attacks felled timber of *Acacia seyal* Del., known as Talh. In any stack of this timber the ravages of the beetle become apparent in a very short time. It is said that firewood loaded on to Nile steamers is reduced to powder before it can be burned, and it is certain that of the volume of *A. seyal* cut annually, a large amount either never reaches its destination or else is cast out as soon as it does. Fortunately, the danger is easily apprehended, for the beetle is not a quiet worker but advertises its presence by an incessant crackling noise and by ejecting white wood dust at a rate that is hardly believable. A branch attacked by the beetle (commonly called the Suss) will, when laid on a table for only a few moments, leave behind it conical piles of frass.

Before attempting any control of the insect it was thought advisable to make investigations as to its method of attack, susceptibility to heat and insecticides and, lastly, its associations with other insects. For this purpose, beetles were introduced into a series of insectaries containing pieces of timber that had been subjected to various treatments, and their actions noted. Logs free from attack were used in all cases and artificially infested at the appropriate time.

Description of *S. senegalense*.

The adult is illustrated in fig. 3. In actual size it is just a quarter of an inch in length and about one-eighth of an inch in width. It has the cylindrical form, abruptly truncated posteriorly, with a cowl-like prothorax covering the head, typical of a Bostrychid beetle. The diagnostic three-bladed antenna is clearly visible under a hand lens. Other features that distinguish this species from others with which it is likely to be associated, are a pair of posterior spines on the elytral declivity, the smaller pair of frontal spines, and the general rugose appearance of both elytra and prothorax, the latter being notably globose. It is uniformly almost black and is a sturdy and very lively beetle that runs fast and flies readily. It is attracted to light and a common intruder in lamp-lit houses.

The larva (fig. 1) is a fat, white, curved grub often found with the beetle when splitting up infested timber. Careful examination will show that it bears thoracic legs, unlike the grubs of Scolytid borers. It is particularly to be remarked that the imagines are found boring in company with the grubs, a state of affairs very different from that of certain other wood-boring beetles where the egg is laid in a crevice or cut vessel in the timber and the grub alone does the boring. This has a bearing on control.

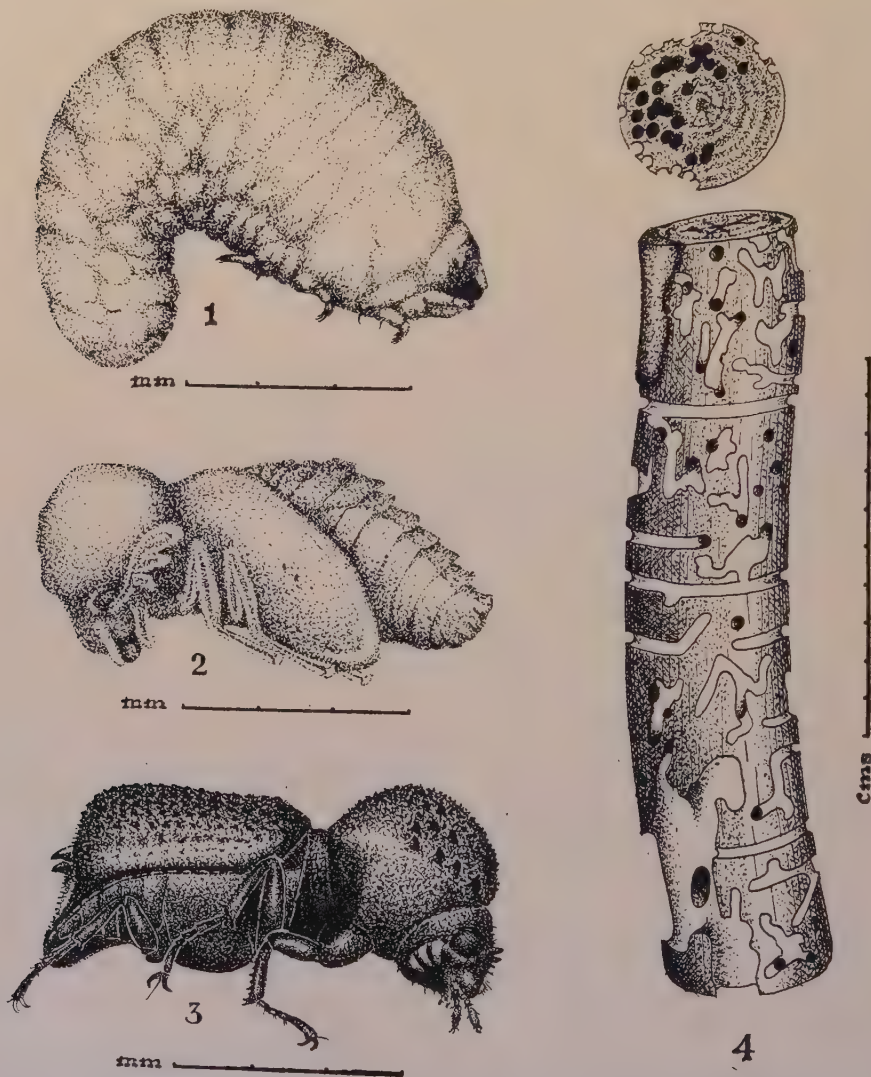
The pupa (fig. 2) is not difficult to recognise as a Bostrychid pupa as it clearly shows the three-bladed antenna of an adult Bostrychid beetle.

Life-history.

Insectary cages containing naturally infested *A. seyal*, which was allowed to remain untouched, gave some indication of the life-history of *S. senegalense* and also provided information on other insects found in this timber. This timber was collected on 1st March, 1951; it was heavily infested and crackled continuously for five weeks

when activity ceased abruptly, with an emergence of hundreds of beetles. The explanation of this was not at the time quite clear, as one would have expected the flying of the beetles to follow a quiescent pupal period of at least some weeks. The presence of overlapping generations would account for the continual activity in the timber up to the time of flight, but not for the silence thereafter. Moreover the wood, when split up and comminuted, was riddled to the core but completely evacuated except for an occasional dead adult which had failed to emerge.

It was evident that the flight of the beetle was a seasonal event from the discovery that logs brought into the laboratory at this time from various sources were also found to be untenanted. It was noted that the fighting beetles showed a very strong



Figs. 1-3.—*Sinoxylon senegalense* Karsch. (1) larva ; (2) pupa ; (3) imago.
Fig. 4.—Talh wood attacked by *Sinoxylon* with Cerambycid damage at the lower end and *Lyctus* damage at the upper.

tendency to cluster in tightly packed numbers in any crevice which afforded them shelter, and in particular in the space between the loose bark and the dead tree. The timber did not in itself appear to be an attraction at this time since tin cans with loose wrappers of brown paper served their purpose just as well. No boring occurred during this period which lasted for one or two days, and the beetles later dispersed, presumably having mated although this was not observed. New logs supplied to the dispersing adults were attacked in the normal way, the beetles boring directly into the timber to oviposit. The logs were cut up eight weeks later and examined. Holes visible from the outside, and from which the outpouring of wood dust had been noticed for a few days after the beetles had been put to the attack, were now found to lead through the bark but not very deeply into the timber. Here, a superficial and irregularly shaped mother gallery, shown in fig. 5, had been excavated and in it were found one or more dead parent beetles. Egg niches were not seen in this oviposition tunnel, but from it larval galleries followed the grain of the timber in an upward or downward direction. These larval tunnels ran closely side by side, all starting from a small restricted part of the mother gallery where the eggs had evidently been laid close together. This resulted in a certain amount of competition, so that only a few larvae pupated where quite a number had started tunnelling.

In this particular experiment the timber had been subjected to the attacks of the beetle exactly two months before. At the time it was cut up for examination, the larvae had just completed their tunnelling and had pupated; some were still in the full grown larval stage while one or two adults had just emerged and were as yet of a light brown colour. It may be fairly assumed, therefore, that the period from egg to adult is about eight weeks. But this is not the complete life-cycle of the insect. The complete life-cycle, being from egg stage to egg stage, comprises also a considerable period when, following eclosion, the beetle tunnels in the wood prior to emergence from it, and this accounts for the boring activity immediately up to the time of flying. This boring is both deep and superficial; indeed it would appear that almost the entire damage is done by the adult insect. In the examination made of the egg chambers and larval tunnels, it was seen that not only were these superficial but also that the larva, in order to complete its growth, required to travel only a few inches whereas the beetle, after eclosion, tunnelled extensively. If there is only one



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Fig. 5.—*Sinoxylon senegalense*. Oviposition tunnels and larval galleries.

swarming in the year (it was recorded from the 7th to the 15th of April) it must follow that the mature wood-boring stage occupies very nearly ten months. With a view to following out this lengthy tunnelling period of the adult beetles, the same logs, in which only the immature stages had been completed over a period of two months, were examined three months later. This five months' old infestation showed the beetle to be still working within the timber, no flighting having taken place. It was not possible to watch these insectaries over a complete year nor, unfortunately, at the time of the next annual swarming, but it appears evident that no intermediate emergence took place.

Nature of Attack.

Freshly cut natural logs.

Freshly cut natural logs were readily attacked immediately after felling although it was noted later that this attack did not appear to have been successful, the beetles burying themselves in the timber and taking no further action. Three months later they were found dead in this position and there was no indication of oviposition or larval tunnels. Whether this is always the case with fresh timber still in sap it is difficult to say, but this is not really of prime importance in the field, where trees cut in the felling areas lie in the sun to become dried out in a very short time. Such trees are very soon found and attacked by the beetle and in this connection it is notable that, in a tour made of the Fung area, isolated trees felled by the road-side and separated by a mile or more, were frequently found to be infested. It happens that the fresh logs cut for this laboratory experiment had a bark greenish rather than reddish, and it is said by some that green timber as opposed to that with red bark, is immune to the attack of the beetle. This seems unlikely since botanically the two types are not separated by any specific or even varietal feature but the observation is worthy of note. All the logs were attacked only on the underside.

Freshly cut barked logs.

Barked logs certainly presented more difficulty to the beetle because, in attacking a piece of natural timber, the insect takes advantage of the separation between bark and wood which always follows on drying. Failing this, or if the bark has been removed, there will generally be a split or axe-cut available but if not, the beetle will often wedge itself in between two contiguous logs and start its excavation from there. Apart from the mechanical advantage offered by the bark, there appears to be no other reason why natural logs should be more readily attacked. Once again it was noted that the beetles entered the logs from the underside.

Old logs.

Old logs, well dried out for some months after felling, barked and unbarked billets being enclosed together, were all attacked as one would expect, this being the usual way in which the beetle is noticed under natural conditions. The only information gained from this experiment was that the logs were again attacked only from the underside.

Vertically placed logs.

Logs placed vertically with their lower extremities in sand had been previously stood on bases of paper, pine-wood, felt and suchlike unpalatable materials to see whether the beetle, finding no under surface, would prefer starvation to an attack from the side or top. The insects solved the difficulty by boring down through the felt or other material and coming up into the logs from beneath. When the logs were stood in sand without disturbance for two months, it was found that the sawn lower surface of the wood was grooved in all directions by the beetles which had burrowed into the

sand in order to gain the lower extremities. There was, however, apparently not enough purchase to enable them to bore upwards. It might at first sight appear that here was a very simple and effective means of control, and there is no doubt at all that if logs, at the time of felling, were immediately barked and stood on end the attack would be reduced to a very low level indeed. The timber would be no sooner sold, however, than it would be carried off and stacked on its side and its destruction would not be long delayed.

Treatment of Logs.

Natural logs soaked in water.

An insectary cage containing natural logs soaked for ten days in water gave indifferent results. Logs so treated are held by the Sudanese merchants to be proof against the beetle. In the Suki sawmill premises there are iron tanks in which the barked logs are soaked for 14 days, and in the Bunzuga nursery a concrete tank is used for the same purpose, the soaking time being put up to three weeks. It is difficult to believe that dipping in cold water for a fortnight or three weeks should prove efficacious, particularly with regard to future attack after drying. It is true that in the present experiment the soaked logs were not attacked but neither were the controls. Once again this may have been due to using the green-barked timber and not the red.

Natural logs soaked in hot and cold sodium arsenite.

Treatment in a hot and cold sodium arsenite dip for one day proved to be effective. It is most important that the timber should be allowed to cool in the solution ; it is quite useless to dip the timber in the hot solution (even for many hours) and then to allow it to cool in the air. Under the latter conditions very little penetration takes place and the timber is sterilised by the heat of the solution but not made immune to future attack. The chemical in solution will penetrate the timber only if the log is transferred rapidly from hot to cold solution or, alternatively, if it is allowed to remain submerged until the hot solution cools. The latter method seems the better suited to our mills as all the tanks can be filled and heated together and there is plenty of time for these to cool off before the next lot of fellings is received.

In this laboratory experiment, the infested timber was subjected to the hot and cold dip system and the chemical, sodium arsenite, was used in concentrations of 1.3 per cent. and 2.6 per cent. by volume. The curious figures of 1.3 per cent. and 2.6 per cent. were arrived at as the volume of one and of two standard sized cigarette tins in an ordinary four-gallon kerosene tin of water which is a simple method of measurement for the workers in the mills. In terms of weight, one cigarette tin of sodium arsenite is (without the tin) three-quarters of a pound, so that three pounds of chemical go to 16 gallons of water. The weaker of the two solutions was found to be effective in the small-scale laboratory experiment. It is rather less than the strength usually used for the purpose but it appeared to be effective. The beetles will attack timber so treated, as they also will with double the concentration, but they do not bore more than half an inch into the wood before the poison has had its effect. Some penetration of the chemical is therefore necessary to prevent the beetles' entry, for sodium arsenite is a poison but not a deterrent. Herein lies the necessity for the hot and cold dip system.

The question of danger to the workmen has been raised in connection with sodium arsenite and advice was sought from the Conservator of Forests, Northern Rhodesia, where the hot and cold sodium arsenite dip has been practised with great success. Mr. A. E. Fitzpatrick replied as follows :—

“For a number of years past, sodium arsenite has been widely used on a considerable scale for treating over two million cubic feet annually in Northern

Rhodesia. If the labourers' hands are protected with gloves and if they wear sandals, or covering for their feet is provided in the area of overspill or drip from the tanks, no harm arises. The Conservator can remember only two accidents over a period of ten years."

Natural logs soaked in a hot and cold boracic solution.

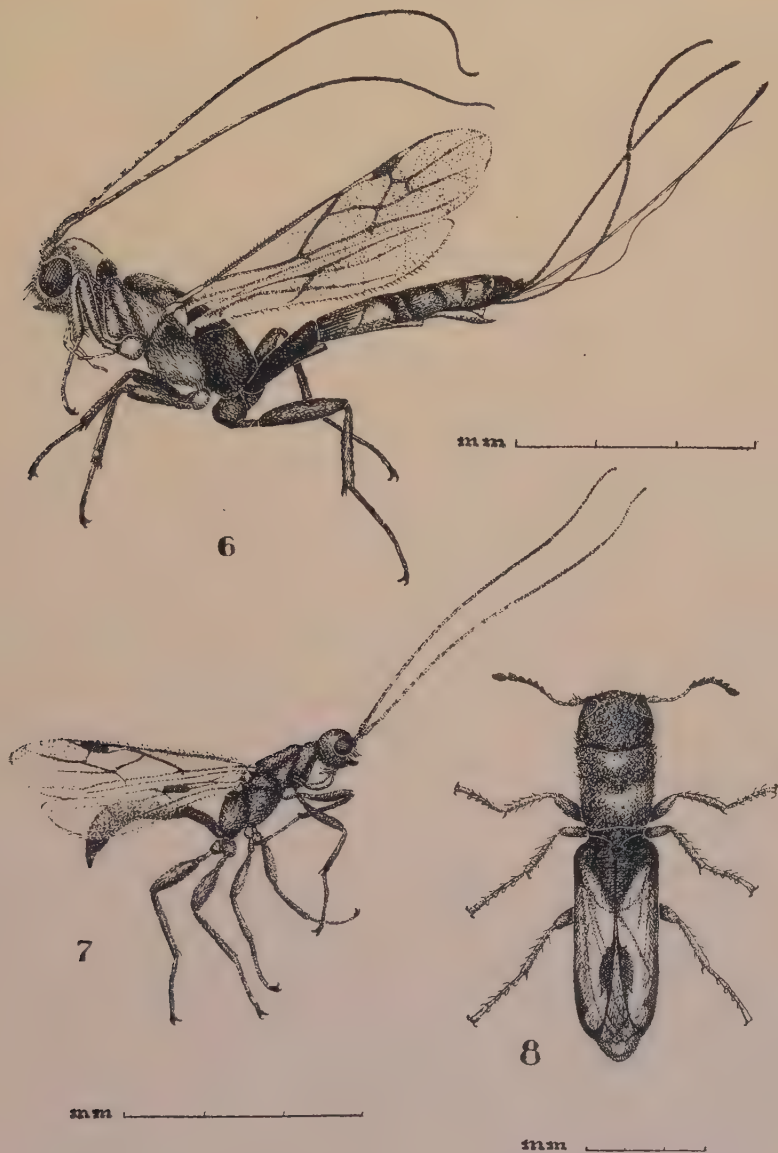
As a completely safe yet effective method of control was insisted upon in the Sudan, a borax dip was prepared for experiment. No useful information could be drawn from infested logs treated for one day in a hot and cold dip of 1.3 per cent. and 2.6 per cent. by volume boracic acid powder in water. Beetles were put into this cage and the results noted a month afterwards. The attack was very slight even on control logs. One of the 1.3 per cent. borax treated logs, however, had been bored and, on splitting up, showed a beetle to have reached a depth of $1\frac{1}{2}$ ins. before death. A disadvantage of the borax dip was the readiness with which moulds grew upon treated timber and indeed on the borax solution itself.

Freshly cut and barked logs sprayed with pentachlorophenol.

Freshly cut logs, barked and sprayed with 5 per cent. by weight pentachlorophenol in a light and penetrating solvent of aromatic oil, represented a means of control which could be applied in the forest. The object would be to prevent the initial attack of the beetle and also to render the timber immune to further attack even when stacked in yards or loaded on steamers. The results of this experiment were remarkably good, the controls being heavily attacked and the treated logs barely nibbled. This pentachlorophenol treatment seems very promising for the protection of the timber provided that it is sprayed in the forest at the moment of felling and barking.

S. senegalense, and many other Bostrychids, bore so rapidly into felled logs that it would be impossible to transport the timber to the mills before eggs have been laid deep in the wood and far beyond the reach of any insecticidal spray. The early application of the pentachlorophenol process is simplified by the portability of a knapsack sprayer and the fact that the logs are, in any case, barked as a routine immediately on felling. What has yet to be learned from field trials is the amount of spray absorbed by freshly cut and barked logs; the costs will depend on this and also on any unforeseen difficulties which may be encountered in the practical application of the method. In the small laboratory experiments the pentachlorophenol was dissolved in a light aromatic oil obtainable commercially as "Shell Talpa 40" which gave remarkably good penetration. The vapour pressure of pentachlorophenol at 30°C. is less than 0.0005 mm. of mercury and its solubility in water less than 0.002 per cent. at the same temperature. These properties, together with its chemical stability, ensure a lasting action in a temperature such as prevails in the Sudan and even in the event of rain. Danger to the workers would appear to be at a minimum provided regard is given to the hazard of fire, a precaution necessary wherever petroleum oils are used.

Fortunately, and a most important consideration where the timber has to be transported by Nile steamer, together with passengers and foodstuffs, there is no persistent smell from treated logs. Sprayed logs lose any smell within a few days (this being the petroleum vapour) and in the case of billets treated in the laboratory it was soon quite impossible to tell, either by colour or smell, treated from untreated wood. The insecticide in some quantity, long since ordered from England, is to be used for field trials in Kassala Province and should provide an adequate pointer to the use of pentachlorophenol in the Sudan. There is, however, one particularly notable point. The treated logs in the laboratory experiments were not proof against *Lyctus*. Whether this is due to the particular method of oviposition or whether to a natural resistance to the insecticide has not yet been established. In either case the presence of *Lyctus* has no practical bearing on the subject since logs of *Acacia seyal*



Figs. 6-7.—A species belonging to a genus near *Doryctes*. (6) ♀; (7) ♂.
 Fig. 8.—*Cylindrus fasciatus* Lap. (Cleridae).

are reduced to powder by *Sinoxylon* long before *Lyctus* has had any appreciable effect. The straighter logs which, after spraying, could be set aside for building huts and not for burning, would no doubt suffer eventually from *Lyctus*, particularly as such poles consist largely of sapwood.

Timbers attacked.

Acacia seyal Del., locally known as Talh, is the principal species attacked by *S. senegalense* in the Sudan. As stated above, the timber is consumed thoroughly from bark to core.

Acacia albida Del., known as Haraz, is also sometimes attacked, the tunnels going deeply into this light timber.

Acacia arabica Willd., the common riverside acacia known as Sunt, grown extensively on plantation scale for timber and firewood, is attacked to some degree but the borings never penetrate deeper than the sapwood.

Other Insects associated with Acacia Logs.

Cerambycids, Lyctids, Buprestids, Elaterids, Tenebrionids, Histerids, Cucujids and a Clerid beetle are all to be found in the massed community of a Talh log being consumed by *S. senegalense*. In one instance only, in a Haraz log infested solely by *S. senegalense*, Braconid parasites appeared. Two species of these emerged, the more common is a species belonging to a genus near *Doryctes*, of which the female and male are illustrated in figs. 6 and 7, and the rarer species is *Platyspathius pictipennis* Viereck.

Of this community on *A. seyal*, the Cerambycids and Lyctids do an estimable amount of damage but could hardly be classed as competitive with *S. senegalense*. Fig. 4 shows the infested log as it appears when the bark is removed. The sculptured appearance is the work of *S. senegalense*, both the irregular tunnels and those which run in a strictly transverse direction. Superimposed on these may be seen the occasional wide excavations of the Cerambycid borer, as shown at the lower end of the billet of wood illustrated in the figure. These latter excavations are in the form of irregularly shaped cavities and associated with them may be seen the large oval hole which represents the deep boring of the same insect. *Lyctus* damage, seen at the top of the billet, takes the form of a compacted mass of frass with faint traces of tunnelling and showing the minute emergence holes of the insect.

Amongst beneficial insects, *Cylidrus fasciatus* Lap. (fig. 8), an easily recognisable Clerid with transparent elytra, probably takes toll of a large number of the tunnelling larvae. It may be found, both in its larval and imaginal forms, in the tunnels wherever these contain *Sinoxylon* larvae and will often emerge from one log, fly to another, and disappear down a deeply penetrating tunnel. At the same time, since *A. seyal* is regularly destroyed by the borers, this active predator may be considered only as a retarding factor in the final dissolution of the timber. Much the same may be said for the parasites which, curiously enough, have emerged only from *A. albida* timber (one instance only) and never from *A. seyal*. Haraz is a soft and light wood, Talh is hard and dense, and it may well be that the latter offers too much resistance to a frail ovipositor. That oviposition is effected through the timber and not by pursuing the host down the tunnels, may be surmised from the faintly serrated stylet of the parasite. Oviposition has never been witnessed and the parasite itself has appeared only once from the many thousands of *Sinoxylon* bred in the insectaries.

Summary.

Sinoxylon senegalense is essentially the wood borer of felled *Acacia seyal* Del. in the Sudan, damage due to other beetles being contributory in a very small degree.

The life-history of the Bostrychid is recorded. The period from egg to adult is about eight weeks, but the complete life-cycle is considerably longer. The larval tunnels are superficial and only a few inches in length. The damage is almost entirely due to the adult, which remains boring within the logs for nearly 10 months. A predacious Clerid, *Cylidrus fasciatus*, and Braconid parasites, a species belonging to a genus near *Doryctes* and *Platyspathius pictipennis*, were recorded.

Destruction of the timber is very rapid and control methods should be undertaken as soon after felling as possible. Insectaries were prepared containing the timber

treated in various ways and the behaviour of the insect noted in each instance. The chemicals used in the experiments were sodium arsenite, boracic acid and pentachlorophenol. The latter, used as a spray on barked logs, was found to be the most effective.

References.

- DUFF, C. E. (1946). The common timber borer of Northern Rhodesia.—J. S. Afr. For. Ass., no. 14, pp. 37–45.
- KARSCH, F. (1881). Die Käfer der Rohlfs'schen Afrikanischen Expedition 1878–79.—Berl. ent. Z., **25**, pp. 41–50.
- KIRKPATRICK, T. W. (1944). Notes on insect damage to East African timbers.—31 pp. Nairobi, E. Afr. War Suppl. Bd, Timber Contr.
- LESNE, P. (1924). Les Coléoptères Bostrychides de l'Afrique tropicale française.—Encycl. ent., (A) **3**, 301 pp. Paris, Lechevalier.
- TOOKE, F. G. C. & SCOTT, M. H. (1944). Wood-boring beetles in South Africa. Preventive and remedial measures.—Bull. Dep. Agric. S. Afr., no. 247, 37 pp.
- VRYDAGH, J. M. (1951). Faune entomologique des bois au Congo Belge. Les insectes Bostrychides (première note).—Bull. agric. Congo belge, **42**, pp. 65–90.
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A GROUPING BY LARVAL CHARACTERS OF SOME SPECIES OF THE GENUS *APANTELES* (HYMENOPTERA: BRACONIDAE).

By J. R. T. SHORT, D.Phil.

University College of Hull.

The genus *Apanteles* is usually divided into four main groups on adult characters (Marshall, 1885; Wilkinson, 1932). The purpose of this study is to examine the larval characters in relation to this grouping of the adults. It was thought desirable to confine the study mainly to Palaearctic species as sufficient material of these species was available. The nomenclature used is that of Kloet and Hincks (1945). The terminology has been discussed by the author (1952) and is outlined in the lettering of fig. 1.

The material was available as cast skins in the cocoons of bred material. British Museum material was used whenever possible and material (determined by Wilkinson) at the Hope Department of Entomology, Oxford, was also used. Preparations were made from the material by the method described by the author (1952).

Preparations made from British Museum material have been deposited at the British Museum (Natural History). The remaining material has been deposited at the Hope Department of Entomology.

The Larval Characters.

The characters which were thought to be useful in grouping the species were the form of the mandible, the number of setae on the prelabium, the number of setae on each maxilla, the degree of sclerotisation of the skin (*i.e.*, whether light, dark or intermediate) and the degree of sclerotisation of the head sclerites. As will be seen in Table I, the nature of the mandible clearly allows groups S and U (of Wilkinson) to be distinguished from groups A and F (of Wilkinson). The form of mandible which is called type I possesses a line of prominent teeth (fig. 1). The form of mandible which is called type II usually does not possess prominent teeth and fig. 2 is of a form with slight teeth. Within type II, two further types can be distinguished, one in the A group and one in the F group. In the A group the mandible (except in *A. formosus* (Wesm.)) has a group of small teeth at the tip (fig. 3). In group F, teeth, if present on the mandible, are situated along the length of the blade (fig. 2). The mandible type I is similar in both group S and group U. It does not appear possible to distinguish between these groups on larval characters.

The remaining characters do not give a means of differentiating groups. They have been recorded since they may be of interest to workers concerned with the identification of larval stages. In group U all species possess a lightly sclerotised skin. In group A all species except *A. triangulator* (Wesm.) possess a lightly sclerotised skin. In group F all species except *A. solitarius* (Ratz.) possess a dark or rather dark skin.

The members of groups S and U approach the genus *Microgaster* in larval characters.

The author (1952) stated that spines are never present on the larval skin of the MICROGASTERINAE. This is correct for species with a lightly sclerotised or moderately sclerotised skin. The skin of all species is covered with small conical projections. In species with a well sclerotised skin the apex of each conical projection is sclerotised and the skin is best described as possessing spines.

TABLE I.

	Type of mandible	Number of setae on prelabium	Number of setae on each maxilla	Appearance of skin and head sclerites	Number of teeth on mandible
Group S					
<i>A. anarsiae</i> F. & A. ...	I	2	1	light	16
<i>A. ater</i> (Ratz.) ...	I	2	1	light	25
<i>A. butalidis</i> Marsh. ...	I	2	1	light	16
<i>A. carbonarius</i> (Wesm.) ...	I	2	1	intermediate	14
<i>A. lacteicolor</i> Vier. ...	I	2	1	light	24
<i>A. obscurus</i> (Nees) ...	I	2	1	dark	20
<i>A. tirathabae</i> Wilk. ...	I	2	1	light	23
<i>A. ultor</i> Reinh. ...	I	2	1	light	18
<i>A. viminetorum</i> (Wesm.) ...	I	2	1	intermediate	18
<i>A. xanthostigmus</i> (Hal.)...	I	2	1	dark	20
Group U					
<i>A. imperator</i> Wilk. ...	I	2	1	light	17
<i>A. laevigatus</i> (Ratz.) ...	I	4	1	light	22
<i>A. longipalpis</i> Reinh. ...	I	2	1	light	22
<i>A. sicarius</i> Marsh. ...	I	2	1	light	16
Group A					
<i>A. formosus</i> (Wesm.) ...	II	4	1	light	
<i>A. inclusus</i> (Ratz.) ...	II	2	1	light	
<i>A. pallipes</i> Reinh. ...	II	2	1	light	
<i>A. triangulator</i> (Wesm.)	II	10	2	dark	
Group F					
<i>A. gabrielis</i> G. & R. ...	II	2	1	intermediate	
<i>A. gastropachae</i> (Bch.) ...	II	2	1	intermediate	
<i>A. glomeratus</i> (L.) ...	II	2	1	dark	
<i>A. gonopterygis</i> Marsh. ...	II	2	1	dark	
<i>A. jucundus</i> Marsh. ...	II	2	1	dark	
<i>A. limbatus</i> Marsh. ...	II	2	1	dark	
<i>A. melitaeorum</i> Wilk. ...	II	2	1	dark	
<i>A. pilicornis</i> Thoms. ...	II	2	1	dark	
<i>A. popularis</i> (Hal.) ...	II	2	1	intermediate	
<i>A. praepotens</i> (Hal.) ...	II	2	1	dark	
<i>A. rubecula</i> Marsh. ...	II	2	1	intermediate	
<i>A. solitarius</i> (Ratz.) ...	II	2	1	light	
<i>A. spurius</i> (Wesm.) ...	II	2	1	dark	
Species of uncertain position					
<i>A. circumscriptus</i> (Nees)	I	14	3	dark	13
<i>A. laetus</i> Marsh. ...	I	12	2	dark	17

Notes on the Groups.

Notes have not been given on the species of groups S and U. All the features which were thought to be useful in identifying the species of these groups are listed in the Table. The number of teeth recorded includes all tooth-like projections on the upper edge of the mandible. Thus the upper half of the bifid tip of the blade of a mandible is considered as a tooth, but not the lower half. With groups A and F the number of teeth (if present) on the mandible cannot easily be distinguished. Descriptions of the mandible, etc., are therefore given as aids to identification.

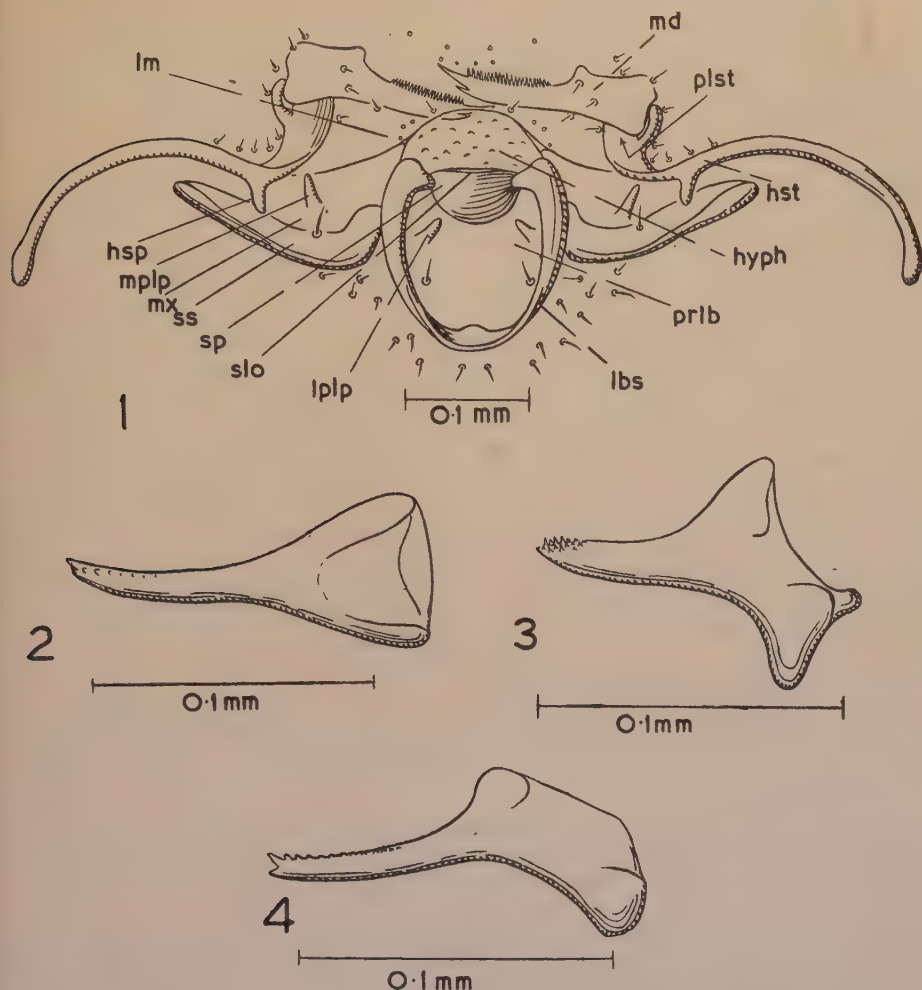


Fig. 1.—Head sclerites and mandibles of *A. tirathabae* Wilk. Abbreviations used : hsp, hypostoma ; hyph, hypopharynx ; lbs, labial sclerite ; lm, labrum ; lplp, labial palp ; md, mandible ; mlp, maxillary palp ; mx, maxilla ; plst, pleurostoma ; prlb, prelabium ; slo, aperture of salivary gland ; sp, silk press ; ss, stipital sclerite.

Figs. 2-4.—Mandibles of : (2) *A. praepotens* (Hal.) ;
 (3) *A. triangulator* (Wesm.) ;
 (4) *A. solitarius* (Ratz.).

Group A.

A. formosus (Wesm.) has a mandible similar in form to that of *A. triangulator* (Wesm.) (fig. 3) but teeth are absent. The setae in the head region are conspicuously long. The seta on the maxilla is equal in length to the mandible.

A. inclusus (Ratz.) has small conical teeth on the apex of the blade of the mandible.

A. pallipes Reinh. has very small, ridge-like teeth on the apex of the blade of the mandible.

A. triangulator (Wesm.) is usually placed in group A on adult characters, but is considered a somewhat aberrant form. On larval characters it clearly falls within group A, but there are ten setae on the prelabium, two setae on each maxilla, the skin is dark and the head sclerites well sclerotised. These characters contrast with those of the remaining members of the group.

Group F.

Within group F, the more pronounced are the teeth on the mandible the more pronounced is the bifid tip to the blade. Forms such as *A. gonopterygis* Marsh., *A. rubecula* Marsh. and *A. solitarius* (Ratz.) (fig. 4) approach groups S and U in the form of the mandible.

Teeth, when present, are situated along the length of the blade of the mandible.

A. gabrielis G. & R. has small ridge-like teeth. *A. gastropachae* (Bch.) has small conical teeth similar to those of *A. praepotens* (Hal.) (fig. 2). *A. glomeratus* (L.) has small conical teeth and the hypostoma posterior to the hypostomal spur is conspicuously sclerotised. *A. gonopterygis* has distinct teeth similar to those of *A. solitarius* (fig. 4). *A. jucundus* Marsh. has very small conical teeth. *A. limbatus* Marsh. does not appear to have teeth on the mandible. *A. melitaeorum* Wilk. has small conical teeth. *A. pilicornis* Thoms. has small needle-like teeth. *A. popularis* (Hal.) has small conical teeth. *A. praepotens* (fig. 2) has small conical teeth. *A. rubecula* has distinct teeth similar to those of *A. solitarius*. *A. solitarius* (fig. 4) has distinct teeth. *A. spurius* (Wesm.) has conical teeth.

Notes on certain aberrant Species.

A. butalidis Marsh., when considered on adult characters, has perhaps more affinity with group S than with any other group. The larval characters clearly indicate group S or group U. The species has therefore been placed in group S in the present account.

A. carbonarius (Wesm.) is, on adult characters, an odd species with apparently no close European ally. It is usually placed in group F. The larval characters however, indicate group S or group U. The species has been placed in group S in the present account.

A. circumscriptus (Nees) is a remarkable form having 14 setae on the prelabium, three setae on each maxilla, a dark skin and well sclerotised head sclerites. The mandible is of type I (groups S and U). On adult characters this species has been placed with group A.

A. laetus Marsh. has features in common with *A. circumscriptus*. The mandible is of type I, although it approaches type II in that the teeth are slender. There are 12 setae on the prelabium and two on each maxilla. The skin is dark and the head sclerites well sclerotised. On adult characters this species is usually thought to be most nearly related to group U. Mr. G. E. J. Nixon's interpretation of the adult characters is that *A. laetus* should be placed with *A. circumscriptus*. The larval characters support this interpretation.

Notes on some other Species.

A. congregatus Say described by Fulton (1940) belongs to group F. The mandible is of type II. There are two setae on the prelabium and one on each maxilla. This species is placed in group F on adult characters.

A. fumiferanae Vier. described by Brown (1946) appears to belong to group S or to group U. The mandible is of type I and there are two setae on the prelabium and one on each maxilla.

A. sesamiae Cam. described by Ulyett (1935) appears to belong to group F, although the teeth on the mandible are larger than is usual in this group. There are two setae on the prelabium and one on each maxilla. On adult characters *A. sesamiae* is often placed within group F. Wilkinson places this species in a small group M.

A. thompsoni Lyle described by Vance (1931) appears to belong to group S or to group U on the structure of the mandible. There are two setae on the prelabium and one on each maxilla. On adult characters this species is often placed with group A.

A. solitarius is figured by Parker (1935). His figure of the mandible suggests group S or group U. However, British Museum material of *A. solitarius* determined by Mr. G. E. J. Nixon has been examined. The characters, which are listed in the Table, indicate that the species belongs to group F, although the teeth are larger than is usual in this group and the tip of the blade is markedly bifid. This species is placed in group F on adult characters.

Summary.

The larval characters of 33 species (largely Palaearctic) of the genus *Apanteles* have been examined and studied in relation to the division of the species of the genus into four major groups on the characters of the adults. In general, the larval characters indicate a grouping of the species which is very similar to the grouping of the adults. Groups S and U contrast sharply with groups A and F on the form of the mandible. The form of the mandible also allows group A to be distinguished from group F. The relationships of four aberrant and some other species are discussed.

I wish to thank Mr. J. F. Perkins of the British Museum (Natural History) and Professor G. C. Varley of the Hope Department of Entomology, Oxford, for permission to study material. Mr. G. E. J. Nixon's advice on the relationships indicated by the characters of the adults of the species of the genus *Apanteles* has been invaluable.

References.

- BROWN, N. R. (1946). Studies on parasites of the Spruce Budworm *Archips fumiferana* (Clem.). 1. Life history of *Apanteles fumiferanae* Viereck (Hymenoptera, Braconidae).—Canad. Ent., **78**, pp. 121-129.
- FULTON, B. B. (1940). The Hornworm parasite, *Apanteles congregatus* Say and the hyperparasite, *Hypopteromalus tabacum* (Fitch).—Ann. ent. Soc. Amer., **33**, pp. 231-244.
- KLOET, G. S. & HINCKS, W. D. (1945). A check list of British insects.—483 pp. Stockport, the Authors.
- MARSHALL, T. A. (1885). Monograph of British Braconidae. Part I.—Trans. ent. Soc. Lond., **1885**, pp. 1-280.
- PARKER, D. L. (1935). *Apanteles solitarius* (Ratzeburg), an introduced Braconid parasite of the Satin moth.—Tech. Bull. U.S. Dep. Agric., no. 477, 17 pp.
- SHORT, J. R. T. (1952). The morphology of the head of larval Hymenoptera with special reference to the head of Ichneumonoidea, including a classification of the final instar larvae of the Braconidae.—Trans. R. ent. Soc. Lond., **103**, pp. 27-84.
- ULLYETT, G. C. (1935). Notes on *Apanteles sesamiae*, Cam., a parasite of the Maize Stalk-borer (*Busseola fusca*, Fuller) in South Africa.—Bull. ent. Res., **26**, pp. 253-262.

- VANCE, A. M. (1931). *Apanteles thompsoni* Lyle, a Braconid parasite of the European Corn Borer.—Tech. Bull. U.S. Dep. Agric., no. 233, 28 pp.
- WILKINSON, D. S. (1932). A revision of the Ethiopian species of the genus *Apanteles* (Hym. Bracon.).—Trans. ent. Soc. Lond., **80**, pp. 301-344.
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THE CONSTRUCTION AND CALIBRATION OF AN ELECTRICAL HYGROMETER SUITABLE FOR MICROCLIMATIC MEASUREMENTS.

By E. B. EDNEY, Ph.D.

Zoology Department, Birmingham University.

The ideal hygrometer for microclimatic work should be very small, sensitive and accurate over the whole range of the humidity scale and able to withstand exposure to saturated atmospheres. It should be capable of giving a continuous record at a distance, have no effect upon the microclimate, and be cheap and easy to manufacture. Such a paragon of an instrument does not, and probably never will, exist, but there have been useful advances during the last decade, and each advance brings the possibility of widening and refining our rather scanty knowledge of microclimatology.

Solomon (1945) described a technique for measuring humidity in small spaces by using pieces of pure cotton fibre paper, impregnated with cobalt thiocyanate, a salt which changes colour from blue to red as the humidity increases. By comparison with standard colours he obtained an accuracy of ± 2 per cent. R.H. over medium ranges, and ± 5 per cent. R.H. at high or low humidities. The only serious objection to the method is that the humidity cannot be read at a distance, neither can it be recorded.

Another useful method is due to K  ie (1948) who used the fact that glass wool adsorbs water vapour, and therefore alters its surface resistivity, to an extent dependent upon relative humidity. Instruments based on this principle can be made as small as 40×6 mm., and with suitable apparatus humidity can be recorded at a distance. There appears to be little objection to this hygrometer in theory except possibly its rather large size for some types of work and the fact that resistances of 1 megohm or more are encountered. In practice, however, the design is inconvenient in so far as the glass wool fibres are on the outside of a glass tube, and are therefore liable to be damaged.

The "Gregory" hygrometer, which is manufactured under patent by Messrs. Negretti and Zambra, is based on the variation in resistance of glass wool yarn impregnated with a hygroscopic salt. The hygrometer described in the present paper derives from the last two methods: it resembles the Gregory hygrometer closely in principle, but is considerably smaller than the standard models. It is approximately the size of a full grown woodlouse. Ansbacher and Jason (1953) have published a preliminary description of what may be a very useful method, based upon the electrical properties of anodised aluminium.

The electrodes of the element (fig. 1, E) are short pieces of 0.04-in. diam. platinum-clad nickel-iron wire. Two of these are mounted in perspex end-pieces (P), and each is connected by flexible insulated copper wire to the measuring instrument (M). A spiral of continuous thread glass-fibre yarn (G) is wound round the two electrodes and impregnated with about 2 drops of a 1.0 per cent. solution of calcium chloride. The electrodes and glass-fibre filaments are protected by four thin rods of non-corrosive wire (W), and the overall dimensions of the elements are then $10 \times 6 \times 6$ mm. When used in the field the element may be further protected by a small wire-gauze envelope.

The resistance of the glass-fibre spiral varies with the humidity of the air to which it is exposed, for the hygroscopic salt adsorbs or releases water as the humidity changes. If now a constant voltage is applied across the electrodes, the current which flows

will vary with the humidity. The measuring (or recording) instrument consists essentially of a micro-ammeter. It is necessary to use alternating current to avoid polarisation of the element, and this is provided through a vibrator (V_i) from a 12 volt D.C. source (12 V). A "Nife" accumulator is convenient. A suitable circuit is shown in fig. 1.

In operation, before a reading is taken, the fixed resistance (5,500 ohms) is brought into circuit, and the potentiometer (500 ohms) adjusted to give a pre-determined reading on the micro-ammeter. This ensures that the input is constant for each reading. The hygrometer element is then brought into circuit in place of the fixed resistance and the micro-ammeter is read. Temperature at the hygrometer element is read at the same time by means of a small thermocouple which is permanently attached to each element.

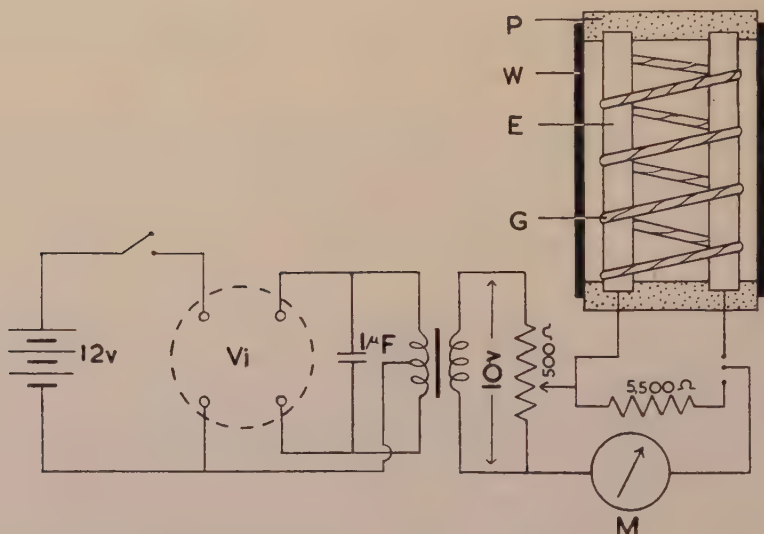


Fig. 1.—The hygrometer element and a suitable wiring circuit. E, platinum-clad electrodes; G, glass-fibre yarn; M, measuring instrument (micro-ammeter); P, perspex end-pieces; V_i , vibrator; W, protecting wires; $1\mu F$, 1 microfarad condenser; 12V, 12 volt D.C. source; 10V, 10 volt A.C. output from transformer; 500 Ω , potentiometer; 5,500 Ω , fixed resistance.

Calibration.

Apparatus.

Before describing the calibration and performance of this type of hygrometer, it must be emphasised that each is an individual instrument. The large elements which are manufactured industrially can be standardised and brought to a given calibration by adding small quantities of the hygroscopic salt. But the elements described here take such small quantities of salt that it is much more convenient to use an approximately correct amount of salt and to calibrate each element individually.

The resistance of the glass fibre and salt varies with humidity; it also varies with temperature at a given relative humidity. Calibration must therefore be carried out at three (or more) different constant temperatures covering the range in which the element is to be used. The process of calibration therefore involves exposure of the element to air at controlled humidities for considerable periods

of time. Calibration can be done over acid and water or KOH and water mixtures (Solomon, 1951) (provided the air is stirred all the time), but in the writer's experience it is difficult to avoid minute droplets of the controlling fluid contaminating the elements, which show signs of damage (at least with sulphuric acid) by a slight browning. In order to avoid all possible contamination an apparatus was constructed in which the elements can be exposed to air containing water vapour only, and by this means more consistent results have been obtained.

The principle of the apparatus is simple : it involves cooling air with a high water vapour content to a known temperature at which the air will be completely saturated with water vapour, and subsequently raising it again to the required calibration temperature, when the relative humidity will be precisely known. Different relative humidities at the necessary calibration temperature can then be obtained by varying the low temperature at which the air is saturated. In practice these conditions are not simple to obtain.

The apparatus is shown diagrammatically in fig. 2. Distilled water in the "humidifier" (Hu) is raised to about 60° or 70° by a heater (H_1) controlled by an energy regulator. No accurate thermostatic control is necessary at this stage. Air is caused to bubble through this water by an air pump (P) and diffuser. Because

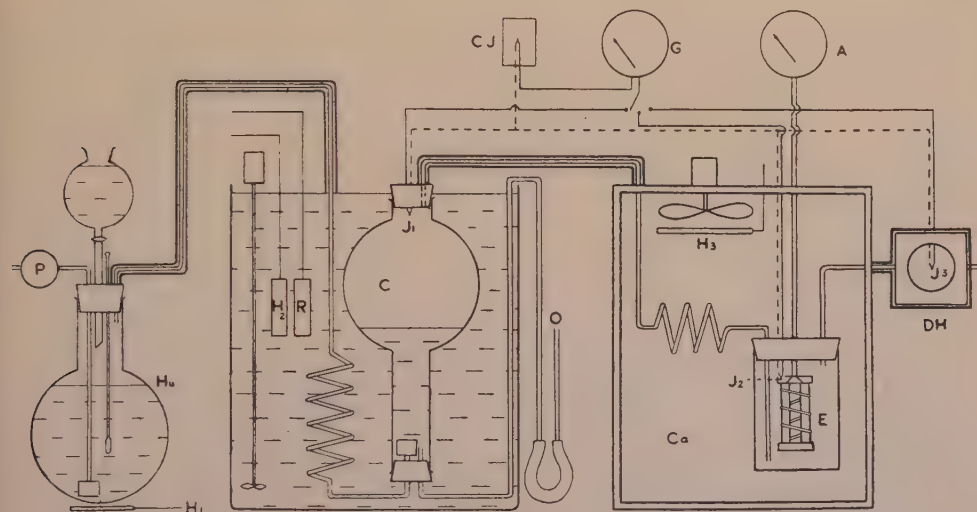


Fig. 2.—Apparatus used for calibration in the laboratory. A, micro-ammeter; C, cooling chamber; Ca, calibration chamber; CJ, cold junction; DH, dew-point hygrometer; E, hygrometer element; G, galvanometer; H_1 , H_2 , H_3 , heaters; Hu, humidifying chamber; J_1 , J_2 , J_3 , thermocouple junctions; O, outlet maintaining constant water level in the cooling chamber; P, air pump; R, refrigerating coil.

the air becomes hot it picks up a large quantity of water vapour and leaves the humidifier by a copper tube which carries it to the cooling chamber (C). When the final humidity required is high, the temperature in the cooling chamber will approach that in the final calibration chamber (Ca), and this may be well above room temperature. It is essential therefore that moist air, when passing from the humidifier to the cooling chamber, shall not fall below the temperature of the latter, for water would then be deposited and the air would not be saturated at the "cold" temperature of the cooler. For this reason, the whole of the tube from humidifier to cooler is heated by a jacket of resistance wire, and this is indicated in the diagram by a thicker black line outside the tube itself.

The cooling chamber is immersed in a large container of water which is stirred and kept at the desired temperature by heating and cooling coils, shown diagrammatically in the figure by H_2 and R. The copper tube carrying the humidified air passes in a long wide spiral to discharge through a diffuser into the cooling chamber itself. This is a large inverted flask, partially filled with water. Much condensation of water occurs in the tube leading to the chamber and in the chamber itself, and the water is maintained at the desired level by the outlet system shown in the figure (O). After passing along the copper spiral and bubbling through the water in the cooling chamber, the air stream is approximately at the temperature of the water in the container, and is quite saturated with water vapour.

The air outlet from the cooling chamber must be carefully constructed, for it is necessary to know accurately the temperature at which the air is saturated. This temperature is measured by a thermocouple (J_1) very near the outlet. The copper tube which carries the air away is also heated, since (for the reason given above) condensation would otherwise occur when working at high humidities. The end of the heated copper tube must not, however, be allowed to cause a rise in temperature of the air inside the cooling chamber, since this would lead to unsaturated conditions near the outlet where the "saturated" temperature is taken. The copper tube therefore ends half-way through the rubber bung.

The air stream is then led to the calibration chamber (Ca). It is convenient to use for this purpose a small incubator which contains a fan and which can be heated (H_3) or cooled to a constant temperature. After passing through a copper coil inside this chamber, the air is discharged into a small glass tube where it passes over the hygrometer element (E). The temperature of the air at the hygrometer is read by a thermocouple (J_2), and leads run from the element itself to a micro-ammeter (A), graduated in units of 10 from 0 to 450 μ amp., outside the chamber.

Now the temperature at which the air is saturated with water vapour is known and the temperature of the air as it passes over the element is known, so that the relative humidity at the element can be calculated from vapour pressure tables. As a check, the dew-point of the air which has passed over the element is determined. This should of course be the same as the temperature of the air as it leaves the cooling chamber, if the apparatus is working satisfactorily. In order to find the dewpoint, a flat mirror dew-point hygrometer (D.H.) (of the type supplied by the firm of Casella) is used, but the temperature of the dew-point is read not by a thermometer in the ether behind the mirror (a method which gives extremely inaccurate results) but by means of a fine thermocouple (J_3) on the surface of the mirror where the dew forms. If the dew-point hygrometer is placed inside the calibration chamber it is difficult to determine the dew-point accurately when high humidities are being used, for the depression is then only a fraction of a degree below the temperature of the air. The difficulty can be overcome by heating the air to well above its dew-point before it passes into the dew-point hygrometer. This in turn means having the hygrometer outside the calibration chamber, in a separate, heated container, and leading the air to it by a short heated duct. This arrangement was finally used and proved very satisfactory, the dew-point being much sharper and easier to determine.

All three thermocouples are wired as shown in the diagram, through one cold junction (CJ) and a three-way switch to a "Scalamp" galvanometer (G) whose sensitivity is approximately 1 cm./0°C.

In practice, the temperature of the calibration chamber is first set at the required level. The desired humidity is then established approximately by setting the temperature of the cooling chamber at the necessary level. After the apparatus has had time to settle down—a process which may take several hours—readings are taken in the following order: temperature of the air leaving the cooling chamber,

temperature at the element, resistance of the element, and dew-point of the air which has just passed over the element. Readings are taken at intervals until three successive readings are the same. A number of elements can of course be calibrated at the same time.

The method described above provides a double check of the humidity of the air as it passes over the hygrometer element, but it was thought advisable, before accepting the dew-point hygrometer readings as valid, to check them in air whose humidity was determined by another method, namely by bubbling through acid-and-water mixtures, made up according to the figures published by Stokes and Robinson (1949). Air was caused to bubble slowly through the mixture and over the dew-point hygrometer in a continuous circuit—the temperature of the whole being maintained constant at 25°C. Dew-point determinations were made, and the humidities derived therefrom were compared with the theoretical humidity determined by the acid water mixtures. Of ten such comparisons, the two measures corresponded precisely in 2 cases, were within 0.5 per cent. R.H. in 6 cases, and within 1.0 per cent. in the other 2 cases. It was therefore assumed that the dew-point determinations could be relied upon to determine R.H. within 1 per cent.

Results.

Turning now to the results obtained with the calibration apparatus, in 31 out of 36 readings, the humidities determined by the temperature of the cooling chamber and subsequently by the dew-point of the air, corresponded within 1 per cent. R.H., and the other 5 readings differed by less than 1.5 per cent. For purposes of constructing the calibration curves, the humidity used was that given by the dew point: this is probably the more reliable of the two estimates, for the other depends upon the assumptions (*a*) that the air is completely saturated at the point where its temperature is measured and (*b*) that it takes up no further water whatsoever when its temperature rises. That these assumptions are in fact valid is rendered likely by the fact that when differences between the two humidity estimates did occur, they were in either direction, *i.e.*, they were reading errors rather than functional faults.

The readings from one element (known as element D) obtained at three temperatures, 25°, 15° and 20°C. (largely in that order) are shown in fig. 3. The numbers which are also entered in the figure indicate the order in which the points were determined, from which it will be seen that at each temperature determinations involved jumping about over the range. The curves connecting the points have been drawn in empirically—there is at present no means of expressing the relation mathematically.

The determination of these points involved a period of three weeks, and it is clear that the element concerned (and the other two which were calibrated at the same time, and which gave similar curves) behaved in a reasonably consistent manner, and this implies that once calibrated, an element can be relied upon not to go badly out of calibration for a fortnight. One or two of the points determined seem to be rather badly off the curve, notably numbers 4, 5, and 8. Examination of corresponding points for the other elements (not shown in the figure) show that they too are correspondingly high or low, and by the same amount. The similarity of the apparent error shown by each element at these points is very striking, and suggests strongly that it is not the elements themselves which erred, but that there was a fault elsewhere in the system which involved all three elements. Such a fault was not traced, but an error in dew-point determination would produce just such an effect.

At relative humidities below about 50 per cent. the curves given by element D begin to flatten out, so that readings below this point become liable to greater errors. It is therefore preferable, if reasonably high accuracy is desired, to use a different element for the lower humidity ranges. An element with more hygroscopic salt

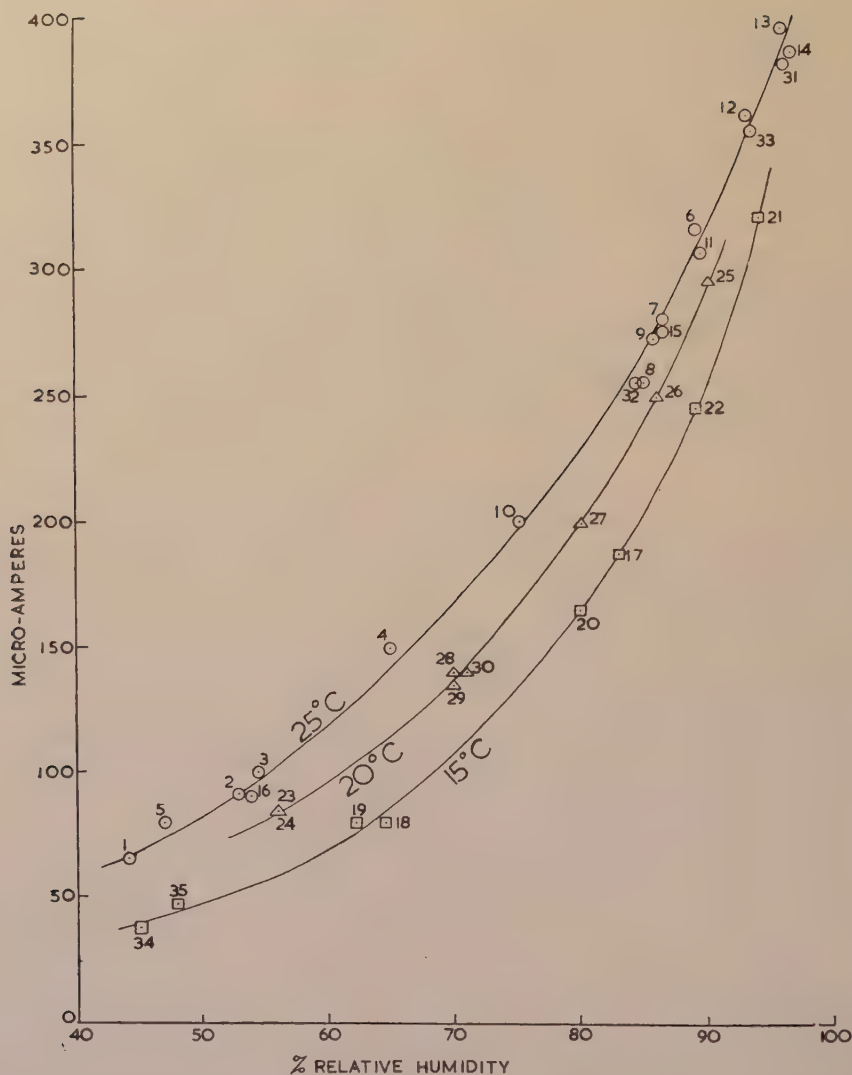


Fig. 3.—Curves obtained with element "D" at 15°, 20° and 25°C. Numbers on the curves indicate the order in which the points were obtained: the last point was obtained three weeks after the first.

gives higher readings at lower humidities, and one of the same size as D, treated with a 5 per cent. solution of calcium chloride, will give satisfactory readings down to 10 per cent. R.H. It will not, however, give satisfactory readings above 60 per cent.

Perhaps the most serious drawback of the hygrometer described is that it cannot be exposed to completely saturated air, since there is a risk of contamination with droplets of liquid water which might remove some of the salt and consequently alter the calibration of the instrument. Humidities as high as 98 per cent., however, can be withstood without affecting the calibration.

Examination of the curves in fig. 3 shows that within the limits mentioned above, the element has behaved reasonably consistently. It must be remembered that the curves have been drawn empirically, but none of the experimentally determined points is off the curve by more than 2 per cent. R.H. It has already been shown above that some of these deviations are likely to be due to errors in estimating the humidity (since all three elements varied consistently at these points), so that it is safe to assume that readings of humidity, obtained by means of these curves, would not be in error by more than ± 2 per cent. R.H. As shown by the order in which the observations were made, the element seems to be remarkably free from hysteresis.

It remains to say something of the equilibration period and stability of the elements. The speed of equilibration was determined by suddenly exposing an element to a new humidity and taking readings every 10 sec. for the first min., every min. for the next 9 min., then at intervals of 10 min. up to 1 hr. Two typical curves obtained in this way are shown in fig. 4, from which it is clear that the greater part of equilibration is accomplished very rapidly, within about 5 min., and that within 10 min. the elements are within a fraction of 1 per cent. of their final reading, which is reached in 20 min. Since an error of ± 1 per cent. is in any case involved in the method of calibration, it would in practice be safe to read the element after 10 min. These determinations were made in the calibration apparatus described above; the element was sealed off in air at a given humidity while the apparatus was set for a new humidity, and once the latter had been obtained the air was again allowed to flow over the element. Air at the new humidity would, of course, take an appreciable time to remove all air at the old humidity from the vessel containing the element, and this would account for the shape of each curve as it leaves the

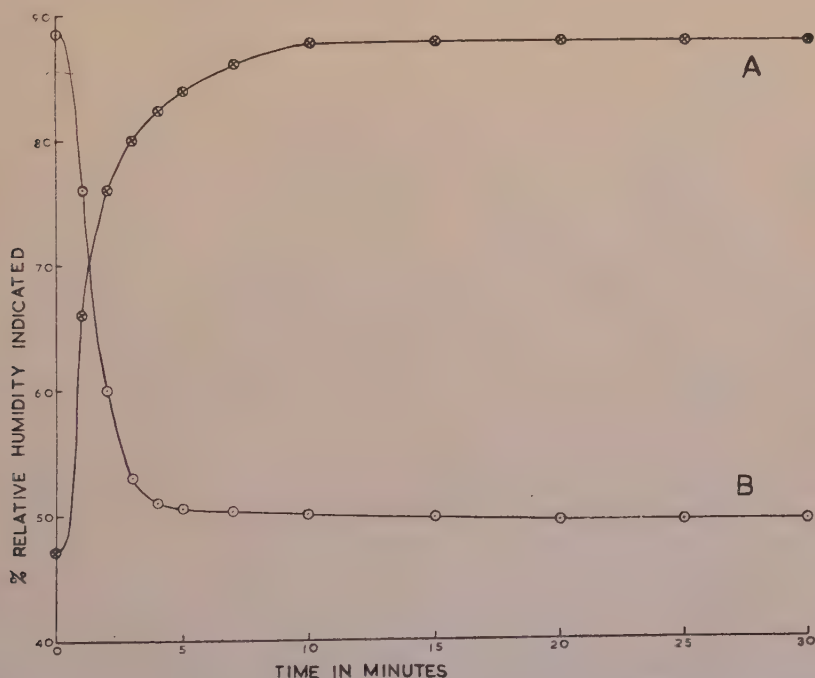


Fig. 4.—Curves showing the time taken for an element to equilibrate to a new humidity. A, element previously kept at 47 per cent. R.H. and exposed to 88 per cent. R.H.; B, element previously kept at 88 per cent. R.H. and exposed to 49 per cent. R.H.

ordinate. No equilibration curves were determined in still air—they would presumably be rather flatter.

As regards stability, experiments show that there is a rise in the resistance at any one humidity as the elements age. The micro-ammeter readings at 80 per cent. R.H. for one element of which records were kept over a period of three months, varied from 220 to 120. The greater part of this change occurred during the first fortnight, and during the last three weeks the change was only from 125 to 120 (it was during these three weeks that the element was calibrated, together with element D whose curves are shown in fig. 3). The process of ageing is very slow unless the elements are subjected to alternating high and low humidities, and it has been found useful to do this once every hour of each day for a week. (The elements can be transferred from air over CaCl_2 to air over water and *vice versa*.)

There is not much change after ageing for three months (providing the element has been subjected to alternating humidities at the commencement of ageing), but there is still the slow drift mentioned above, and it is this drift, which is not altogether regular, which makes it necessary to re-calibrate an element every fortnight or so.

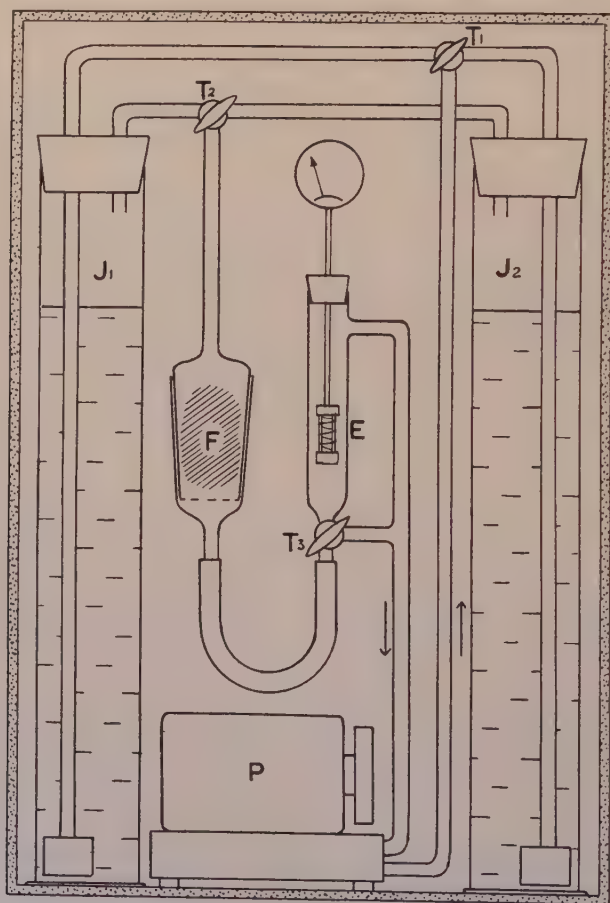


Fig. 5.—Portable apparatus for re-calibration away from the laboratory. E, hygrometer element; F, cotton wool filters; J₁, J₂, jars containing acid-and-water mixtures; P, continuous circuit air pump; T₁, T₂, T₃, controlling taps.

Use and Calibration in the Field.

It has already been said that the elements are individual research tools. Unfortunately they cannot easily be standardised, and each must be calibrated separately. When in constant use calibration should be carried out about once a fortnight, and it would detract seriously from the value of the instrument if its calibration had to be done in the elaborate apparatus described above. Fortunately this is not necessary, for although an element will not stand exposure to air over acid for long periods, it is perfectly possible to expose them for a short time without any harm at all. Once the general shape of the curve has been determined, variations therefrom can be corrected by determinations made at a few points, and since the elements settle down quickly, only short exposures (about 15 min.) are necessary. A convenient, portable apparatus for re-calibration is shown in fig. 5. It consists essentially of a circulation of air through acid-and-water mixtures and over the element. The mixtures are held in tall jars so that a hydrometer can readily be used to check the specific gravity of the acid, and thus the humidity of the air, immediately before and after a reading. The apparatus is designed with two independent circuits, so that determination at two different humidities can be made rapidly and without disturbing the apparatus.

Air emerging from the pump (P) is directed by the tap (T_1) through the acid-and-water mixture in either jar (J_1, J_2), and from there through the tap (T_2) to a filter (F) which consists of a B29 ground glass cone and socket containing a fresh pad of cotton wool. The air stream is then directed by the tap (T_3) either straight back to the pump (while the air is coming into equilibrium with the acid-and-water mixture) or over the element (E) and then back to the pump. The air-temperature is measured by the thermocouple attached to the element. The whole apparatus is enclosed in a wooden case and is easily portable.

Conclusions.

The instrument described above does not possess all the qualities of an ideal hygrometer mentioned at the outset, but it does have certain advantages for microclimatic work. It is small, it can be read or recorded at a distance and there is no continuous evaporation of water such as is inevitable with any form of wet bulb hygrometer. For this reason, too, there is very little disturbance of the natural environment.

For use in the field away from a laboratory, all the equipment can be carried in a suit case: a micro-ammeter and 12 volt D.C. source is necessary, also a galvanometer and cold junction (thermos flask) for use with the thermocouple. A preliminary account of certain measurements made in the field has been published (Edney, 1952), and a preliminary account of the hygrometer itself was read to the IXth International Congress of Entomology at Amsterdam in August, 1951.

Summary.

The paper describes the construction, calibration and performance of an electrolytic hygrometer based on the principle of the "Gregory" hygrometer, but considerably smaller. Its dimensions are about $6 \times 6 \times 10$ mm.

The element consists of two platinum-clad nickel-iron electrodes, holding a spiral of continuous fibre-glass wool yarn which is impregnated with a solution of calcium chloride. The water content of this hygroscopic salt varies with humidity, so that the resistance between the two electrodes also varies. In order to measure this resistance, alternating current of a known voltage is used, and the current is measured in terms of micro-amperes. A suitable circuit is described. Air temperature is measured by a fine thermocouple permanently attached to each element.

Long exposure to air above acid and water mixtures damages the elements so that calibration is best carried out without acid. An apparatus in which this can be done is described. A stream of air with a high water vapour content is cooled to a precisely known temperature which is below its saturation point, and then raised to the temperature required for calibration, so that its relative humidity is known as it passes over the element being calibrated. Finally the dew-point is again found as a check. Temperature affects the resistance of the elements, and calibration must therefore be carried out at three different constant temperatures.

Curves obtained by means of this apparatus show that one element will give readings from 50 to 98 per cent. R.H. within ± 2 per cent. Another element with more hygroscopic salt must be used for humidities between 60 and 10 per cent. R.H.

There is an ageing process whereby the resistance of each element increases for some time after it has been constructed. This period can be shortened by exposing the elements to alternating high and low humidities for a few days. Elements which have received this treatment can be calibrated and used after six weeks, and will remain in calibration for at least a fortnight.

Subsequent calibrations can be made in a simple apparatus (which is described) involving acid-and-water mixtures, but the use of filters and exposure of the elements for no more than 15 minutes avoids damage.

The hygrometer equilibrates in a new humidity within ten minutes.

The instruments possess certain advantages: they are small, they can be read or recorded at a distance, and they do not depend upon evaporation of water, so that disturbance of the natural environment is minimised. The main disadvantages are that they cannot be exposed to saturated air without going out of calibration and that each instrument must be calibrated individually—they cannot easily be standardised for mass production.

Acknowledgements.

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References.

- ANSBACHER, F. & JASON, A. C. (1953). Effects of water vapour on the electrical properties of anodised aluminium.—*Nature*, **171**, p. 177.
- EDNEY, E. B. (1952). Body-temperature of Arthropods.—*Nature*, **170**, pp. 586–587.
- KØIE, M. (1948). A portable alternating current bridge and its use for micro-climatic temperature and humidity measurement.—*J. Ecol.*, **36**, pp. 269–282.
- SOLOMON, M. E. (1945). The use of cobalt salts as indicators of humidity and moisture.—*Ann. appl. Biol.*, **32**, pp. 75–85.
- SOLOMON, M. E. (1951). Control of humidity with potassium hydroxide sulphuric acid, or other solutions.—*Bull. ent. Res.*, **42**, pp. 543–554.
- STOKES, R. H. & ROBINSON, R. A. (1949). Standard solutions for humidity control at 26°C.—*Industr. Engng Chem.*, **41**, p. 2013.

A REVISION OF THE GALL-MITES (ACARINA, ERIOPHYIDAE)
OCCURRING ON TOMATO (*LYCOPERSICUM ESCULENTUM* MILL.),
WITH A KEY TO THE ERIOPHYIDAE RECORDED FROM SOLANACEOUS
PLANTS.

By K. P. LAMB, M.Sc., F.R.E.S.

*Plant Diseases Division, Department of Scientific and Industrial Research,
Auckland, New Zealand.*

In recent years, certain species of gall-mites occurring on tomato plants have become pests of major economic importance, especially in Australia and the U.S.A.; for example, according to Bailey and Keifer (1943), the tomato russet mite was by far the most serious arthropod pest of tomato in California. Some confusion exists concerning the taxonomic status of the various mites concerned and the object of this paper is to throw some light on this problem.

Historical and Discussion.

Nalepa (1929, p. 143) listed the following records of Eriophyids on tomato :—

“*Lycopersicum esculentum* Mill.—Behaarte Auswüchse auf den Stengeln :

E(riophyes) calacladophora Rolfs

Rolfs, Bull. 91 Florida Agric. Stat. 1907 nt. 91 p. 14 (*Phytoptus* c.)

Wolffenstein, Monatsch. Gartenb. 1879 v. 22, p. 424 (*Phytoptus lycopersici*).

Lycopersicum sp.—Tomato Rosette Disease and Fruit Sterility :

Phyllocoptes lycopersici Tryon

Tryon, Rep. Queensland Agric. Dep. 1916–17, p. 49–63.”

1. *Erineum* Mite.

Wolffenstein (1879) described *Phytoptus lycopersici* which he stated was causing a disease of tomato called “ceniza” (Aschenkrankheit) in southern Spain. As symptoms of plant damage, he mentioned the greyish-white appearance of infested plants resulting from abundant, abnormal hair growth (erineum) on leaves and stems and also degeneration of the plant accompanied by greatly diminished flower production.

In 1939, Massee described another species, *Eriophyes lycopersici*, attacking tomato in Morocco. He stated that it produced patches of abnormal hairs on stems, leaf stalks or other parts of the plant in addition to causing stunting and crop reduction.

Though the morphological detail given by Wolffenstein in his description of *Phytoptus lycopersici* is insufficient for detailed comparison with *Eriophyes lycopersici*, the following facts suggest that the two are synonymous :—

- (1) Gross dimensions of the bodies of both species are similar (*i.e.*, length, and ratio of length : breadth in the female).
- (2) Both species have relatively long first ventral setae. In *P. lycopersici* Wolff., the length of these setae is said to be over half that of the caudal setae. In *E. lycopersici* Massee, the first ventral setae = 50 μ , and the caudal setae = 66 μ .

- (3) The host symptoms associated with their presence are identical.
- (4) The areas from which the two species were described are in close geographical proximity. *P. lycopersici* Wolff. was described from southern Spain and *E. lycopersici* Massee from Rabat, Morocco.

The genus *Phytoptus* Dujardin 1851 (*s.l.*) is synonymous with *Eriophyes* v. Siebold 1850 (*s.l.*) (Nalepa 1898, 1911). Keifer (1944) restricted the use of *Phytoptus* Duj. 1851 (*s.s.*) and split the genus *Eriophyes* into the genera *Eriophyes* v. Sieb. 1850 (*s.s.*) and *Aceria* Keifer 1944. The mites of the genus *Aceria* are separated by having the dorsal setiferous tubercles on the rear shield margin and directing the setae caudad. The location of the dorsal shield setae places the above species in the genus *Aceria* and, as it appears that only one species is involved here, its name now becomes *Aceria lycopersici* (Wolffenstein) (**new combination**).

Rolfs (1893) described a disease of the tomato which was caused by an Eriophyid mite "*Phytoptus* sp." and which was characterised by the production of "a white fuzzy outgrowth on the plant." He stated that this condition was called "ashy" in southern Spain. Almost certainly he was alluding to the condition discussed by Wolffenstein and attributed by him to *Phytoptus lycopersici*. In 1898, Rolfs described the disease in greater detail and added: "a disease which is doubtless the same as has been reported from Spain and Italy." He attributed the condition to *Phytoptus calacladophora* Nal. There appears to be no description of the mite under this name and Nalepa did not refer to it in any lists of species which he had described. In 1907, Rolfs redescribed the plant symptoms and attributed them to *Phytoptus calacladophora*. This reference was cited by Nalepa (1929) who attributed the name to Rolfs. However, in the absence of a published taxonomic description of the mite the name must be regarded as a *nomen nudum*. Possibly it was confused with *Phytoptus cladophthirus* Nal. which had been described from *Solanum dulcamara*. In later Bulletins of the Florida Agricultural Experiment Station, Watson gave the name as *Eriophyes calacladophora* and later as *Eriophyes cladophthirus* (Watson, 1914, 1942). Keifer (1946) listed the mite as *Aceria cladophthirus* (Nal.). There is every reason to believe that Wolffenstein (1879) Rolfs (1893, 1898, 1907), Watson (1914, 1942) and Massee (1939) were all considering the same disease of the tomato and that the causative agent was the same species of mite in all cases. This mite should now be known as *Aceria lycopersici* (Wolff.).

2. Russet Mite.

Massee (1937) indicated that *Phyllocoptes lycopersici* Tryon was a *nomen nudum* and described the species under the same name, *i.e.*, as *Phyllocoptes lycopersici* Massee, 1937. He gave as symptoms of infestation: "Causes silvering and curling of the lower leaves: the stem and leaf stalks turn brown and the lower leaves and flowers fall off, and the fruits become stunted and rough skinned. 'Tomato rosette disease and fruit sterility' (Tryon)." The mites were described from specimens collected in Auckland, New Zealand and this may be regarded as the type locality.

Keifer (1940b) described another species of Eriophyid mite on *Lycopersicum esculentum* as *Phyllocoptes destructor*. He stated: "The mites feed on the leaves, stems and fruit, causing severe browning and curling of the leaves and russetting of the fruit." While he recognised that this species was very similar to *Phyllocoptes lycopersici* Massee, he listed the following differences:—

<i>P. lycopersici</i>	<i>P. destructor</i>
"Female 200 μ long	Female 150–180 μ long
Center of shield smooth	Center of shield with discernible lines
Featherclaw 3-rayed	Featherclaw 4-rayed
Female genital coverflap smooth	Female genital coverflap furrowed "

Concerning *Phylloptes lycopersici* Masee and *Phylloptes destructor* Keifer, Bailey and Keifer (1943) were not prepared to admit that these two names applied to one species. However, in 1946 Keifer transferred *P. destructor* to the genus *Vasates* Shimer 1869 and remarked : " The name *destructor* may have to be sunk in favour of *lycopersici* Masee ".

The following investigation was carried out to determine the systematic position of the tomato russet mite which occurs in the Auckland district.

In order to measure the variation of morphological characters, a series of local mites were measured. These were taken from different localities and from two hosts, tomato (*Lycopersicum esculentum* Mill.) and cape gooseberry (*Physalis peruviana* L.). The mites were collected in August, November and January. Thus, variation due to locality, host and season was represented. The measurements were treated according to the methods of Cazier and Bacon (1949) and the variations of measurements of individual characters were compared graphically. No differences were observed between the measurements of mites from the above two host plants, so both sets of measurements were combined. Fig. 1 shows the data derived from measurements of 20 female specimens mounted in Berlese fluid by the method of Keifer (1940a). These data are amplified in Table I.

TABLE I.

Dimensions of New Zealand tomato russet mites (measurements of 20 specimens).

Code	Character	Mean (μ)	Standard Deviation	Coefficient of Variation	<i>P. lycopersici</i> Mass.	<i>P. destructor</i> Keif.
A	Length of body ...	197.4 \pm 6.5	29.01 \pm 4.6	14.7 \pm 2.3	200	150-180
B	Width of body ...	63.2 \pm 1.27	5.7 \pm 0.9	8.7 \pm 1.3	40	55
C	Length of shield ...	44.9 \pm 2.02	9.27 \pm 1.47	20.6 \pm 3.26	44	40
D	Width of shield ...	60.8 \pm 1.19	5.34 \pm 0.85	8.3 \pm 1.31		47
E	Distance between shield setae	44.0 \pm 1.19	5.3 \pm 0.84	12.4 \pm 1.90		37
F	Length of dorsal setae ...	13.95 \pm 0.25	1.1 \pm 0.17	8.5 \pm 1.34	14	13
G	Length of lateral setae ...	24.15 \pm 1.16	5.19 \pm 0.82	21.5 \pm 3.4	20	30
H	Length of ventral setae I	61.75 \pm 1.19	5.3 \pm 0.84	8.5 \pm 1.34	64	60
I	Length of ventral setae II	20.2 \pm 0.70	3.12 \pm 0.49	15.4 \pm 2.23	12	16
J	Length of ventral setae III	26.25 \pm 0.87	3.87 \pm 0.61	14.8 \pm 2.34	20	24
K	Length of caudal setae ...	65.0 \pm 1.35	6.03 \pm 0.95	9.3 \pm 1.47	74	
L	Length of genital setae ...	14.3 \pm 0.63	2.8 \pm 0.44	19.6 \pm 3.10	30	14
M	Length of thoracic setae I	6.9 \pm 0.42	1.89 \pm 0.30	27.4 \pm 4.34		
N	Length of thoracic setae II	17.45 \pm 1.12	5.02 \pm 0.79	28.8 \pm 4.55		
O	Length of thoracic setae III	34.7 \pm 1.69	7.56 \pm 1.20	21.6 \pm 3.42	30	
P	Width of epigynium ...	25.2 \pm 0.53	2.38 \pm 0.38	9.4 \pm 1.49	24	25
Q	Length of epigynium ...	14.85 \pm 0.42	1.9 \pm 0.30	12.8 \pm 2.02		14
R	Length of fore leg ...	35.15 \pm 0.63	2.8 \pm 0.44	8.0 \pm 1.27	34	35
U	Length of fore-leg tarsus	7.85 \pm 0.27	1.19 \pm 0.19	15.2 \pm 2.40	4	7.5
V	Length of fore-leg tibia ...	7.95 \pm 0.21	0.94 \pm 0.15	11.9 \pm 1.88	7	8
W	Length of hind-leg ...	31.4 \pm 0.56	2.48 \pm 0.39	7.9 \pm 1.25	30	31.5
Z	Length of hind-leg tarsus	7.3 \pm 0.26	1.16 \pm 0.18	15.9 \pm 2.52	4	7.5
*	Length of hind-leg tibia...	6.35 \pm 0.20	0.89 \pm 0.14	14.0 \pm 2.21	6	6

For each character, the left vertical line in fig. 1 represents the observed range of measurements and the right vertical line the calculated population range. The mean is represented by a horizontal line intersecting both vertical lines. The measurements given in the original descriptions of *P. lycopersici* and *P. destructor* are also shown in fig. 1. Variation may be attributed to two sources : firstly, the natural morphological variation inherent in all biological material, and secondly, variation due to experimental error.

All measurements were carried out on unstained objects under an oil-immersion lens; variation due to experimental error could therefore be important. In fact, it was found to be greater with some measurements than with others.

The coefficient of variation of the length of the ventral thoracic setae was very high. It is difficult to measure these accurately as they are inserted at right angles to the body surface and often appear curved and non-planar in mounts. The length of the dorsal shield is another variable character. In this case, the problem is to measure the length of a curved organ inclined at an angle to the longitudinal axis of the body. Since the body is relatively soft-skinned and flexible, its gross dimensions may vary with different mounting techniques. This point is discussed further below.

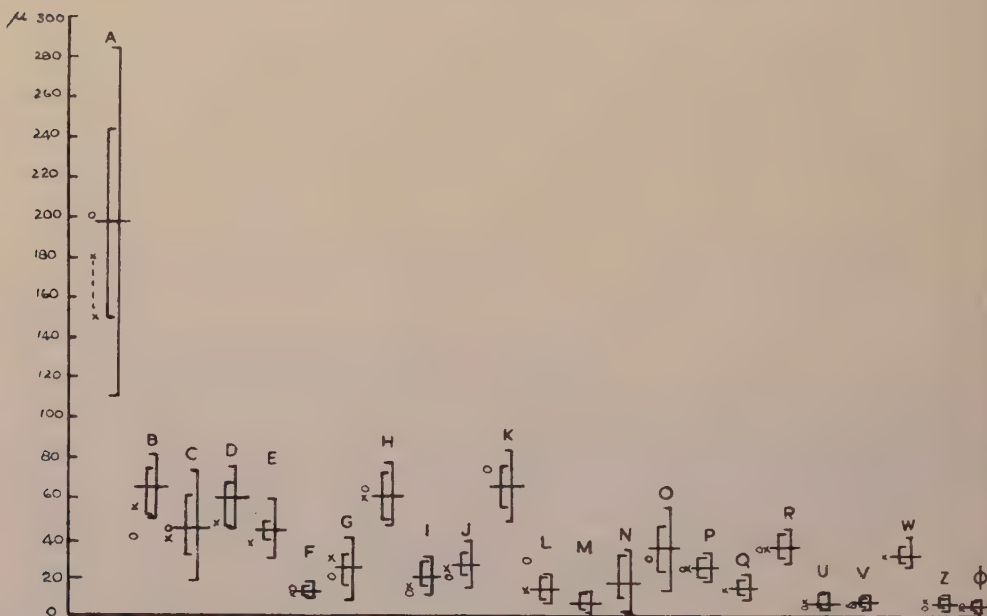


Fig. 1.—Standard data on New Zealand tomato russet mites compared with measurements given in original descriptions of *Phyllocoptes lycopersici* Mass. (shown O) and *Vasates destructor* (Keif.) (shown X). (For key to letters designating sets of measurements, see Table I.)

From fig. 1 it is apparent that all Keifer's dimensions for *P. destructor* fall within the observed range of variation of the measurements of the local mites. The local material was found to agree in all respects with the published description of *P. destructor* (e.g., the 4-rayed featherclaw, the type of striation on the dorsal shield and the genital coverflap, etc.). Examination of spirit material from Davis, California, revealed no differences in structure between the species present in North America and that in New Zealand. Similar symptoms of plant damage are recorded for both species. It may therefore be assumed that they are identical.

With two exceptions, all the dimensions given by Massee (1937) for *P. lycopersici* fall within the observed or calculated range of variation of the dimensions found for the local mite population. The two exceptions are the body width, which is somewhat low, and the length of the genital setae, which is high in Massee's description. The measurements given by Keifer and the author were all taken from Berlese-fluid mounts. Massee, however, was dealing with spirit material and the method generally used by him (private communication) involves mounting in caustic-potash solution.

In order to determine what effect this had, some local mites preserved in 70 per cent. alcohol were mounted in caustic-potash solution and examined 18 hours later. The body width of the ten specimens was on the average $13\ \mu$ less than was found with mites mounted in Berlese fluid. Massee's low estimate of the body width could be explained by the difference in mounting method. No difference was found in length of genital setae of mites mounted in Berlese fluid or caustic-potash solution. If Massee's species is the same as the local one, the difference could be attributed to the measurement of an aberrant individual or to an inaccurate measurement.

The following points arise in a consideration of the differences between *P. destructor* and *P. lycopersici* tabulated by Keifer (1940b) and reproduced above. The difference in body-length of the female is not a valid distinction as both measurements fall within the normal range of variation. The shield striation is less obvious in potash mounts. The striation of the genital coverflap was not observed in 25 per cent. of the mites mounted in Berlese fluid or in 50 per cent. of the mites mounted in potash. When the coverflap is raised at right angles to the body, the pattern is invisible. It was found that when mites preserved in alcohol were mounted in potash, the genitalia protruded and as often as not the coverflap was raised. This was obvious in laterally mounted specimens but was not always apparent in those mounted dorsoventrally. Consequently, the coverflap might appear smooth when in fact it was not. The differences noted in shield- and coverflap-striation could therefore be attributed to differences in mounting technique. Finally, the differences in featherclaw structure must be considered. These organs are notoriously difficult to resolve in detail. They are small ($6\ \mu$ long), three dimensional and complex in structure. Careful examination of local material showed the featherclaw in all specimens to have four rays on each side comprising three stout, compound, lateral rays and one arm of the very slender, simple, bifurcated tip. The illustration in Bailey and Keifer (1943) shows how easily this could be interpreted as three-rayed instead of four-rayed.

The question now arises whether or not *P. lycopersici* described by Massee is the same species as the local material considered in this paper. The original description by Massee was based on material from Auckland, New Zealand, and these mites caused the same type of plant damage as did those now under consideration. Taking into account differences of mounting technique, the only definite morphological difference is the length of the genital setae. The featherclaw structure, which is almost sub-microscopic, need not be taken into account. It is the opinion of the writer that even if Massee's dimension for the genital setae is correct, this difference alone is not sufficient to merit separation of another species. If it were, one could only suppose that the species occurring in this area in 1937 has since been replaced by another species with similar habits and host preference and producing identical symptoms. This appears somewhat improbable. Since the local species is apparently identical with both *P. lycopersici* Massee and *V. destructor* (Keifer), then these must be synonymous, and the proper designation is: *Vasates lycopersici* (Massee) 1937 (**new combination**).

The present status of the gall-mites occurring on tomato may now be summarised as follows:—

Vasates lycopersici (Massee) 1937 (**new combination**).

syn. *Phyllocoptes lycopersici* Tryon, 1917 (*nomen nudum*).

Phyllocoptes lycopersici Massee, 1937.

Phyllocoptes destructor Keifer, 1940.

Vasates destructor (Keifer), 1946.

Aceria lycopersici (Wolffenstein) 1879 (**new combination**).

syn. *Phytoptus lycopersici* Wolffenstein, 1879.

Phytoptus calacladophora ascr. Nalepa, 1898 (*nomen nudum*).

Phytoptus calacladophora Rolfs, 1907 (*nomen nudum*).
Eriophyes calacladophora Watson, 1914 (*nomen nudum*).
Eriophyes lycopersici Massee, 1939.

In order to facilitate comparison of these species with the others occurring on solanaceous plants, the records of other gall-mite species found on such plants are summarised below, and a key is provided for their separation.

LIST OF ERIOPHYIDS FROM SOLANACEAE.

The following additional Eriophyid species have been recorded from plants of the family Solanaceae :—

Aceria eucricotes (Nalepa) (**new combination**).

syn. *Phytoptus eucricotes* Nalepa 1892, Denkschr. Akad. Wiss. Wien, **59**, p. 533.

Phytoptus lycii Canestrini 1892, Atti Ist. veneto, (7) **3**, p. 837.

Eriophyes eucricotes (Nalepa) 1898, Das Tierreich, Lief. 4, p. 34.

Aceria eucricotes (Nalepa) var. *multistriatus* (Kendall) (**new combination**).

syn. *Eriophyes eucricotes* (Nalepa) var. *multistriatus* Kendall 1929, Psyche, **36**, pp. 296–312.

Aceria cladophthirus (Nalepa).

syn. *Phytoptus cladophthirus* Nalepa 1892, Anz. Akad. Wien, **29**, p. 16
 (descr. nulla).

Phytoptus cladophthirus Nalepa 1892, Denkschr. Akad. Wiss. Wien, **59**, p. 526.

Eriophyes cladophthirus (Nalepa). Nalepa 1898, Das Tierreich, Lief. 4, p. 35.

Aceria cladophthirus (Nalepa). Keifer 1946, J. econ. Ent., **39**, p. 570.

Aceria cladophthirus baliotes (Nalepa) (**new combination**).

syn. *Eriophyes cladophthirus baliotes* Nalepa 1921, Treubia, **2**, pp. 146–153.

TABLE II.

Host plants and geographical distribution of Eriophyid species recorded from solanaceous plants.

Eriophyid species	Host Plants	Distribution
<i>Vasates lycopersici</i> (Massee)	<i>Lycopersicum esculentum</i> Mill. <i>Solanum nigrum</i> L. <i>S. nodifolium</i> <i>S. tuberosum</i> L. <i>S. villosum</i> <i>Datura ferox</i> L. <i>D. stramonium</i> L. <i>Physalis ixocarpa</i> Brot. <i>Petunia</i> sp.	New Zealand, Australia, United States of America, Hawaii, Spain (?), Ceylon.
<i>Aceria cladophthirus</i> (Nal.)	<i>Solanum dulcamara</i> L.	Europe, Britain, Russia.
<i>Aceria cladophthirus baliotes</i> (Nal.)	<i>Solanum indicum</i> L.	Java.
<i>Aceria eucricotes</i> (Nal.)	<i>Lycium afrum</i> L.	Europe, North Africa, Canary Is., United States of America.
<i>Aceria eucricotes</i> var. <i>multi-</i> <i>striatus</i> (Kend.)	<i>Lycium halmifolium</i> Mill.	United States of America.
<i>Aceria bicornis</i> (Trotter)	<i>Solanum eleagnifolium</i> Cav.	Argentina.
<i>Aceria lycopersici</i> (Wolff.)	<i>Lycopersicum esculentum</i> Mill.	N. and S. Africa, Spain, Italy, U.S.A. (Florida), West Indies.

Aceria bicornis (Trotter) (**new combination**).

syn. *Eriophyes bicornis* Trotter 1900, Bull. Soc. ent. France, **11**, p. 224.

The host plants and geographical distribution of the Eriophyids occurring on Solanaceae are summarised in Table II.

KEY TO ERIOPHYIDAE OCCURRING ON SOLANACEOUS PLANTS.

1. Abdominal rings differentiated into broad, smooth, dorsal half-rings and narrow, tuberculate, ventral half-rings. (Subfamily : Phyllocoptinae).....
Vasates lycopersici (Masee)
- Abdominal rings uniformly microtuberculate and not dorsoventrally differentiated. (Subfamily : Eriophyinae).....2
2. With a pair of prominent, slender, horn-like structures arising in the anterior half of dorsal thoracic shield.....*Aceria bicornis* (Trotter)
- Without a pair of prominent, slender, horn-like structures arising in the anterior half of dorsal thoracic shield.....3
3. Featherclaw 3-rayed.....*Aceria lycopersici* (Wolffenstein)
- Featherclaw with more than 3 rays.....4
4. Featherclaw 5-rayed.....5
- Featherclaw 4-rayed.....6
5. About 60 abdominal rings with sparse tubercles.....*Aceria eucricotes* (Nalepa)
- About 80 abdominal rings with finer, more abundant tubercles.....
Aceria eucricotes (Nal.) var. *multistriatus* (Kendall)
6. About 70 abdominal rings ; sternum obscurely forked, rostrum small..... :
Aceria cladophthirus (Nalepa)
- About 58 abdominal rings ; sternum simple, rostrum larger.....
Aceria cladophthirus (Nal.) var. *baliotes* (Nal.)

Summary.

The records and descriptions of Eriophyid mites hitherto described from tomato are discussed. A statistical examination was made of morphological measurements of the New Zealand tomato russet mite. The names of gall-mites occurring on tomato have been reduced to two : *Aceria lycopersici* (Wolffenstein) 1879, the tomato erineum mite (causing the production of white, hairy patches on stems and leaf stalks) ; and *Vasates lycopersici* (Masee) 1937, the tomato russet mite (causing stem and leaf bronzing). A key is given to the species of gall-mites occurring on solanaceous plants and several new combinations are recorded.

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References.

- BAILEY, S. F. & KEIFER, H. H. (1943). The tomato russet mite, *Phyllocoptes destructor* Keifer : its present status.—J. econ. Ent., **36**, pp. 706-712.
- CAZIER, M. A. & BACON, A. L. (1949). Introduction to quantitative systematics.—Bull. Amer. Mus. nat. Hist., **93**, pp. 343-388.
- KEIFER, H. H. (1940a). Eriophyid studies VIII.—Bull. Calif. Dep. Agric., **29**, pp. 21-46.
- KEIFER, H. H. (1940b). Eriophyid studies X.—Bull. Calif. Dep. Agric., **29**, pp. 160-179.
- KEIFER, H. H. (1944). Eriophyid studies XIV.—Bull. Calif. Dep. Agric., **33**, pp. 18-38.
- KEIFER, H. H. (1946). A review of North American economic Eriophyid mites.—J. econ. Ent., **39**, pp. 563-570.
- MASSEE, A. M. (1937). An Eriophyid mite injurious to tomato.—Bull. ent. Res., **28**, p. 403.
- MASSEE, A. M. (1939). A species of gall-mite (Eriophyidae) injurious to tomato.—Ann. Mag. nat. Hist., (11) **3**, pp. 617-619.
- NALEPA, A. (1898). Eriophyidae (Phytoptidae).—Tierreich, Lief. 4, 74 pp.
- NALEPA, A. (1911). Eriophyiden. Gallenmilben.—Zoologica, **61**, pp. 167-293.
- NALEPA, A. (1929). Neuer Katalog der bisher beschriebenen Gallmilben, ihrer Gallen und Wirtspflanzen.—Marcellia, **25**, pp. 67-183.
- ROLFS, P. H. (1893). Enemies of the tomato.—Bull. Fla agric. Exp. Sta., no. 21, pp. 23-24.
- ROLFS, P. H. (1898). Diseases of the tomato.—Bull. Fla agric. Exp. Sta., no. 47, pp. 143-144.
- ROLFS, P. H. (1907). Tomato diseases.—Bull. Fla agric. Exp. Sta., no. 91, p. 14.
- TRYON, H. (1917). Report of the Entomologist and Vegetable Pathologist.—Rep. Qd Dep. Agric., 1916-17, pp. 49-63.
- WATSON, J. R. (1914). Tomato insects, etc.—Bull. Fla agric. Exp. Sta., no. 125, pp. 57-78.
- WATSON, J. R. & TISSOT, A. N. (1942). Insects and other pests of Florida vegetables.—Bull. Fla agric. Exp. Sta., no. 370, 118 pp.
- WOLFFENSTEIN, O. (1879). *Phytoptus lycopersici* W.—Monatsschr. Ver. Beförd. Gartenb., **22**, pp. 424-426.
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STUDIES OF *LYGUS VOSSELERI* POPP. (HETEROPTERA, MIRIDAE) A PEST OF CULTIVATED COTTON IN EAST AND CENTRAL AFRICA.

I. A METHOD FOR BREEDING CONTINUOUS SUPPLIES IN THE LABORATORY.

By Q. A. GEERING, M.A.

Empire Cotton Growing Corporation, Cotton Research Station, Namulonge, Uganda.

(PLATE VII.)

Damage to cotton by a species of *Lygus* has been recognised and studied in Uganda since Hancock first described the symptoms in 1935. Originally, the Mirid responsible was known as *L. vosseleri* Popp., but it was later synonymised with *L. simonyi* Reut. Taylor (1947) then revised the East African species of *Lygus* and showed that the cotton species is in fact *L. vosseleri* Popp. Accounts of investigations of this pest in Uganda include publications by Hargreaves (1934), Gwynn (1940), and Taylor (1945). These cover a wide field of investigation into the effects of the feeding of *Lygus* on the cotton plant, and the bionomics of it in the field. The exact status of *L. vosseleri* as a pest has yet to be defined, however, and the study of its biology is still far from complete. It is hoped that the present paper will be the first of a series reporting a complete study of the insect in the field and the laboratory. Taylor's paper (1945), besides detailing his own observations and experiments, reviews many further lines of investigation arising from his own and previous workers' results. These include notably a closer study of the nature of *Lygus* damage, and thence a search for resistance. In order to pursue such investigations intensively, a continuous supply of insects would be required, and these would have to be produced in the laboratory, the period during which adults and nymphs are available in the field in large numbers being extremely limited. Consequently, when work began at the new research station of the Empire Cotton Growing Corporation at Namulonge in Uganda, this aspect of the *Lygus* problem was one of the first to be studied.

This paper describes the development of a method for the continuous breeding of *L. vosseleri* in the laboratory, and includes some information on the biology of the species obtained in the course of this work. The difficulties encountered are detailed, and ways in which it is hoped to improve the technique, and to apply it to the breeding of other species, are outlined.

There is very little published information on the life-history of this pest which could suggest a possible method of breeding. Hargreaves (1934), in an early report, says the condition of the cotton plant is important for oviposition, very soft tissues being essential, and that the distal quarter of the petiole and the base of young bolls are favoured sites for egg-laying. The eggs, as is characteristic of this group of insects, are actually buried in these tissues. Gwynn (1940), in the section on cotton pests in "Agriculture in Uganda", states that "the life-cycle is short, the duration of the nymphal stages being about 14 days". Although the egg is figured in this publication, there is no further reference to it in the text. However, in the original draft, Gwynn states that "while the eggs have never been found in the field, in the laboratory they have been laid in the petioles of young leaves of *Vigna* and cotton, close to the lamina in each case". He describes the egg, giving measurements, and produces evidence to suggest that the larva on hatching is probably vermiform for a very short time. The length of nymphal development quoted above appears to refer to a small number of nymphs reared on cowpea, *Vigna sinensis* L.

Selection of a suitable Medium for Breeding.

The list of the known host-plants of *L. vosseleri* given by Taylor (1945) includes *Sorghum* spp., and Finger Millet, *Eleusine coracana*. He states, furthermore, referring to the Eastern Province of Uganda, that "*Eleusine* and sorghum . . . are major host plants of the insect when the grains are nearly ripe. An enormous population builds up rapidly by breeding on these two crops, . . .". He also adds that "There is very little tendency for *Lygus* to spread from the grain fields so long as the grain is left undisturbed, and any cotton which may be sown abnormally early, remains unattacked throughout this period." It was evident, therefore, that these two grain crops are particularly suited to *Lygus*, as true host-plants, and promised to provide material for rearing nymphs. Whether they could also be used for obtaining eggs was uncertain, for it was not known exactly where on these plants eggs would be deposited.

A wide range of sorghum types and a quantity of *Eleusine* were therefore sown, with a view to testing these as media for breeding. While these were maturing, experiments were started for obtaining eggs in large numbers on other host-plants, the adults used being obtained in the field by sweeping.

Egg production on various media.

The particular difficulty in obtaining eggs from this type of insect is that they are normally deposited within living plant tissue. A dual problem was therefore posed, *viz.*, to find either a material on which the adults could both feed and lay eggs, or failing this, two materials, one for each function.

A first essential was to find a simply prepared food medium for keeping the adults alive. Seedlings of *Eleusine*, *Sorghum*, maize, and cotton were all tested by germinating the seeds on moist cotton wool in glass tubes, and confining the adults with these. The adults fed on the radicles and hypocotyls of all these seedlings, and remained alive for up to three weeks if given a continuous supply of freshly germinated seeds. Their feeding caused a complete disintegration and maceration of the tissues of the radicles, but in the cotton seedlings this effect was less pronounced nearer the hypocotyl, and in this region the tissues were not severely damaged, although the adults did in fact feed here. In addition to feeding, the females also deposited eggs in the radicles of the grain seedlings and in the radicles and hypocotyls of cotton seedlings. Those eggs laid in the damaged radicles failed to hatch, however, partly through the subsequent growth of fungi on the damaged tissue, while eggs laid in the undamaged hypocotyls of cotton seedlings all hatched successfully.

The next step, therefore, was to germinate cotton seeds in a soil medium, and cage the adults with the seedlings. This was immediately successful, eggs being deposited in the upper region of the hypocotyl, and also, if the seedlings were kept under shaded conditions so that the cotyledons diverged considerably, eggs were deposited into the upper surface of the petioles. The adults fed primarily on the apical-bud and growing-point region, but also on the hypocotyls, and on the petioles and laminae of the cotyledons. Feeding on or near the apical bud caused severe damage to the seedlings, and in order to reduce their period of exposure to adults to a minimum, they had to be grown simply, rapidly, and in a way which enabled them to be handled easily and individually. This was achieved in the following manner.

The mineral vermiculite proved an ideal medium for growing the seedlings. With water only, and no nutrient materials added, cotton seedlings will grow in this, and remain healthy for four weeks. The procedure, therefore, was to plug one end of each of a large number of glass cylinders (measuring 4×1 in.) with half an inch of plaster of paris, to fill these with vermiculite, and stand them in water. The vermiculite absorbs water to the top of the tube, and the seeds are sown in the top quarter-inch. Grown thus, and if kept in shade, the seedlings are ready for use within six days. They then bear only the cotyledons on an exceptionally elongated

stem. The growing of seedlings in the plugged glass tubes has a great advantage in that all parts can more easily be examined under the microscope, for making accurate counts of the eggs laid.

By constructing oviposition cages of the type illustrated in fig. 1 *a* and Plate VII, fig. 1, it was possible to change the seedlings without handling the *Lygus* adults, which are extremely active creatures and difficult to handle. Each cage consists of a cylinder of celluloid sheeting, $4\frac{1}{2}$ ins. high by 4 ins. diameter, with a removable muslin cover. The cylinder fits on to a circular base cut from pressed cardboard $\frac{1}{8}$ in. thick. In this, four holes are cut, through each of which a glass cylinder (4×1 in.) containing a cotton seedling can be introduced. The base of the cage is supported on the rim of a coffee-tin (diameter $3\frac{1}{2}$ ins.) whose sides are cut down to 3 ins. Rather less than an inch of each tube thus protrudes into the cage, the plugged end resting on the bottom of the tin which contains 1 in. of water. As each cage is thus set up, the required numbers of male and female *Lygus* are introduced and the cloth cover secured by a celluloid ring clip. The seedlings can then be changed as often as desired, by withdrawing each in turn and inserting a new one, through the holes in the base board.

Once this method had been devised, the production of a continuous supply of eggs (and therefore nymphs) was possible, and it was started with the first adults

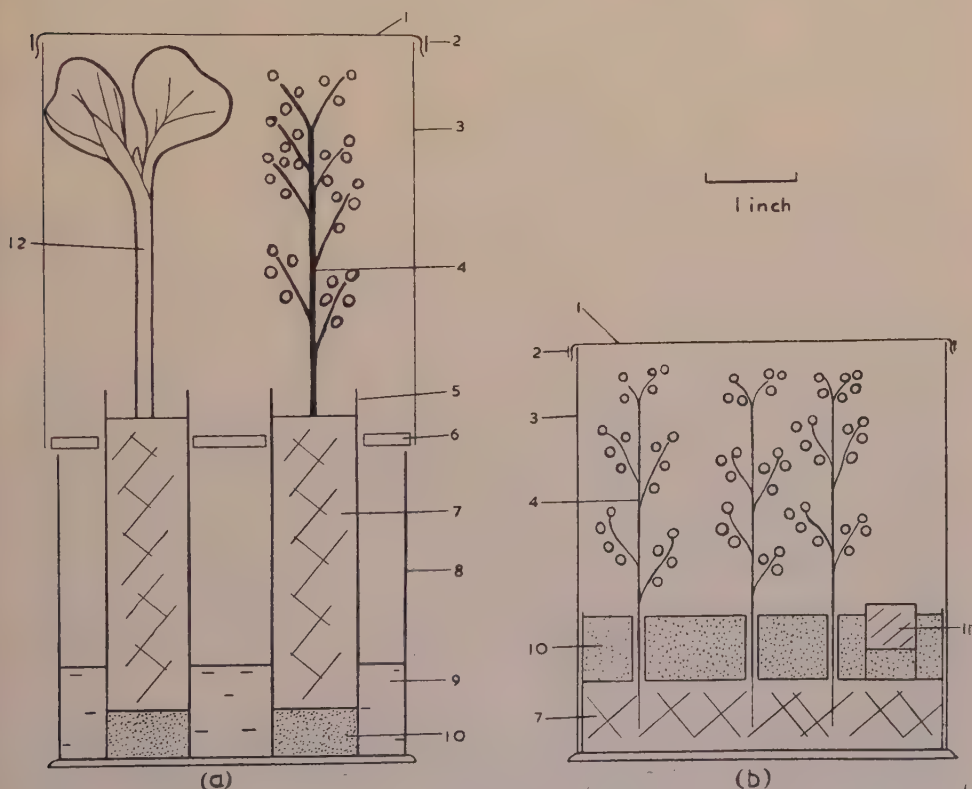


Fig. 1.—Diagrammatic sections of (a) oviposition and (b) rearing cages. (1) Cloth cover; (2) celluloid ring; (3) celluloid cylinder; (4) stem from sorghum panicle; (5) 4×1 in. glass cylinder; (6) $\frac{1}{8}$ in. pressed cardboard, 4 in. diameter; (7) flaked vermiculite; (8) coffee-tin; (9) water; (10) plaster of paris; (11) $\frac{1}{2}$ in. cork; (12) cotton seedling.

bred in the laboratory. It was apparent, however, that although the seedlings were eminently suitable for oviposition, they were less satisfactory when used also as a food for the adults. The chief drawback, indicated above, was the damage they suffered from the feeding of the adults. This invariably included the killing of the apical bud, a feeding site obviously favoured by the adults. Frequently the cotyledonary petioles were also so severely damaged that the cotyledons and growing point died completely. In these two regions, 65 per cent. of the eggs were laid, and they failed to hatch if the tissues containing them were killed. In an effort to reduce this damage, alternative food for the adults in the form of young, milky sorghum grains was supplied as soon as it became available. This was easily accomplished in the cages; instead of growing a cotton seedling in a tube, one or two branches from a sorghum panicle were stuck into the moist vermiculite, and introduced with the seedlings.

Immediate results were a reduction in damage to the seedlings and an increase in longevity of the adults. There was no increase in the total numbers of eggs laid in the seedlings; in fact, the eggs counted in these occurred at more irregular intervals than when seedlings alone were used. In addition, the adults were observed to spend a great deal of time feeding and resting on the sorghum grains. When these were examined after 3-4 days in the cages, eggs were found inserted into them, with the caps showing on the surface. These eggs hatched successfully, and tests were then made for determining the age of grain best suited for oviposition, the sorghum grains being used as the medium for both adult food and oviposition.

Sorghum grains as a medium for egg-production.

The stages in the development of a sorghum grain can be arbitrarily distinguished as follows:—

- (a) The period from fertilisation during which the grain enlarges to its final volume.
- (b) A period during which the grain is fully enlarged and the contents remain "milky".
- (c) A period during which the grain becomes progressively drier, when the sugary contents of the endosperm are being converted to starch.
- (d) The final maturation when the seed dries out, and is ripe.

Tests with grains in stages (a) and (b) were performed as follows. Clusters of grain were cut from sorghum heads grown in the field, and were then exposed in the oviposition cages for 3-4 days. Thence they were removed and placed in hatching cages, and daily counts were made of the first-instar nymphs that emerged. As soon as the grains are cut from the panicle, they begin to dry out, and the stems were therefore placed in moist vermiculite in the cages in order to minimise this effect. These tests showed that the milky grains were the most satisfactory for breeding. The criterion of suitability was taken as the number of first-instar nymphs produced per female. This was decided on for two reasons; firstly, without dissecting out individual grains it is impossible to make an accurate count of the eggs laid, and secondly the success of breeding obviously depended not only on the production of eggs but also on hatching.

The eggs take from 8 to 12 days to hatch, and the period from cutting of the grains to the emergence of the last nymphs was thus 15-16 days. Even under moist conditions, therefore, the grains, though collected in the milky stage, became extremely dried and withered before hatching was complete. It is known that for some Mirids that lay their eggs under similar conditions (Wigglesworth, 1944), there is a period during which the eggs take up water from the plant tissues in which they are embedded. It seems likely that the same factor operates here, and that the greater suitability of the milky grains lies in their slower drying out, the eggs thus

being enabled to absorb sufficient moisture for the completion of development. In this connection it is worth recording that one egg was found lying freely on the surface of a grain; this was kept on filter paper moistened with distilled water, and the nymph emerged successfully at the end of a normal period of development.

Experience has shown that the period in the process of maturation of the grain during which it is suitable for breeding *L. vosseleri* is very limited. Under Uganda conditions, and with the dwarf varieties of sorghum used, the optimum age of grain is from 16 to 20 days after the emergence of the panicle from the sheath. Grains only 2-3 days older than this, *i.e.*, in stage (c), while being suitable for oviposition at the time of collection, subsequently dry out and harden rapidly, and the eggs fail to hatch. Younger grains, *i.e.*, in stage (a), wither and dry out equally rapidly, with subsequent death of eggs. Examination of samples of the grains of different ages used in these tests showed far fewer eggs laid in the very young than in the milky grains.

No thorough examination of grains of different ages has been made in the field; eggs have been found, however, in grains in the same milky stage as those used in the laboratory. Under natural conditions, they are probably suitable for successful egg-development for much longer than is possible once they are removed from the panicle.

The proportion of eggs hatching from the grains is not yet known. The intrinsic fertility of eggs seems normally to be very high, for where all eggs could be counted, as when laid in cotton seedlings, the only ones that failed to hatch were those embedded in tissue that died and dried out as a result of either feeding or the mechanical damage caused by the process of oviposition. In the hatching cages there is considerable interference by saprophytic fungal growths on the sorghum grains. These can be so bad as to prevent whole batches of eggs from hatching. In an attempt to reduce this interference, samples of grains were dipped in a solution of a proprietary copper fungicide, and then dried. This was very effective in reducing fungal growths, but as grains so treated consistently produced fewer nymphs, presumably through the killing of eggs, this type of treatment was abandoned, and the interference by fungal growths is still a major factor in reducing nymphal production.

The effect of the adults' feeding on the grains is to cause them to dry out more rapidly, with a consequent deleterious effect on the hatching of eggs. Employing the sorghum grains for both adult food and oviposition therefore necessitated the determination of the optimum ratio of grain numbers to adults for a given exposure period. It was to be expected that with a higher ratio there would be less chance of grains containing eggs being fed upon. This is partially demonstrated by the results of a test to select an optimum ratio of males to females, and total adults to grains, which are shown in Table I. All variants were placed with 200 grains for alternate periods of 3 and 4 days.

TABLE I.
Test for optimum ratio of males to females, and total adults to grains.

Treatment	Males	Females	Total adults	Total nymphs in 43 days' exposure	Nymphs per female per day	Nymphs per adult
1	2	2	4	215	2.5	54
2	3	3	6	118	0.9	20
3	4	4	8	152	0.9	19
4	3	5	8	392	1.8	49
5	4	6	10	170	0.7	17
6	3	7	10	334	1.1	33
7	2	8	10	378	1.1	38

Within each treatment the variation in nymph production in successive exposures was very great, mainly on account of interference by fungi. Treatment 4, however, was consistently highest, while treatment 1, with a ratio of 50 grains per adult, was clearly the most efficient. The comparative inefficiency of treatments 6 and 7 may have been due either to a lack of males or to the interference of feeding with the hatching of a potentially greater egg supply. When the large-scale breeding was started, therefore, the oviposition cages were set up with 400 grains each and with three males and five females, thus giving the same ratio of sexes as in treatment 4 and the same ratio (50 : 1) of grains to total adults as in treatment 1.

Rearing nymphs.

Before supplies of sorghum grains were available, attempts were made to rear nymphs on cotton seedlings in the same stage of development as those used for obtaining eggs. First-instar nymphs were caged singly on individual cotton seedlings, and their development noted. Where nymphs were left on a single seedling, none survived beyond the third instar. They fed on all parts of the seedling, but again the site most favoured for feeding appeared to be the apical bud, which was invariably killed. If, when this happened, a fresh seedling was provided, the nymph continued to develop, but it became apparent that each nymph would require, even in its early instars, one such seedling per day in order to obtain the apparently essential food contained in the apical bud. As soon as possible, therefore, sorghum grains were tested for rearing the nymphs. The results were so successful that attempts to rear nymphs in large numbers on cotton seedlings were abandoned, and all large-scale rearing has since been carried out on young sorghum grains.

Outline of the method of breeding, and summary of results.

The scheme for continuous breeding was eventually started in January, 1952, when 14 oviposition cages were available. Five females are maintained in each of these, the number of females in use for egg-production thus being kept constant at 70. Twice each week the sorghum grains are changed, dead adults replaced and the old grains set aside in hatching cages. The daily emergence of nymphs has been recorded, and the total production from successive exposures noted. All nymphs from each batch are placed in groups of 40 in rearing cages, and supplied with fresh young grains every two days. Grains in stages (b) and (c) are equally suitable for successful nymphal development.

The hatching and rearing cages are identical, and are illustrated in fig. 1 *b* and Plate VII, fig. 1. The base consists of a shallow tin 4 ins. in diameter by $1\frac{1}{2}$ ins. deep, which is painted with bituminous paint to prevent rusting. Vermiculite is placed in the bottom to a depth of 1 in., and this is covered with a half-inch layer of plaster of paris. Small holes are made in the plaster into which the stems bearing the sorghum grains are inserted, and are pushed down into the vermiculite. This is kept moist with water which can be added through a hole in the plaster, normally plugged with a half-inch cork. It is necessary to keep the surface of the plaster dry in order to reduce fungal growths on the grain; this is done by painting the surface with colourless brushing "Duco". These bases are regularly sterilised in an autoclave and have withstood the treatment satisfactorily. The walls of the cages (which are removable) are made from thin celluloid sheet, and a muslin top is clipped on.

The total production of first-instar nymphs, and adults, over the period of five months from January to May inclusive is shown in fig. 2. This illustrates three features worthy of comment.

(a) Production of first-instar nymphs.

The maximum rate of production so far achieved occurred during the ten-day period from 20th to 29th January. The daily emergence of first-instar nymphs averaged

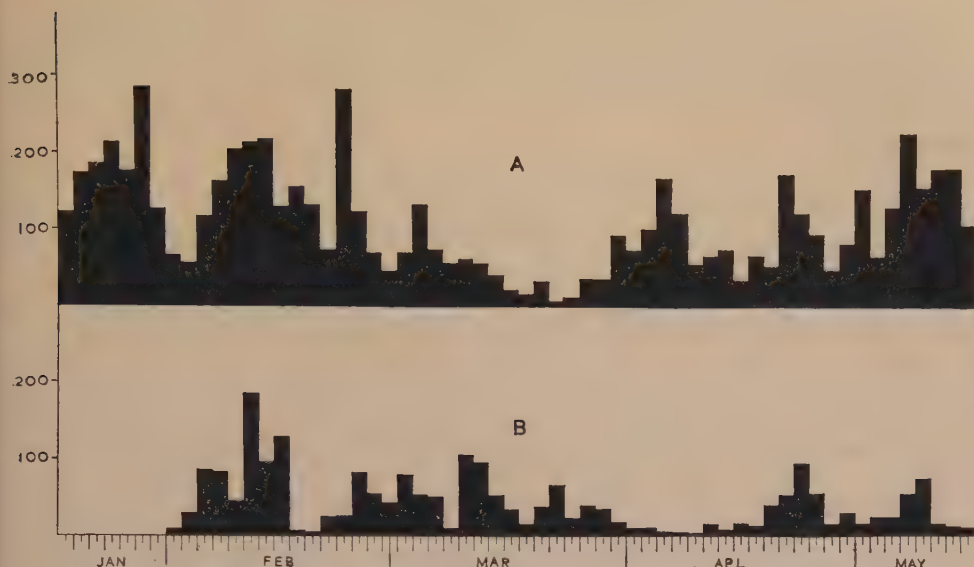


Fig. 2.—*Lygus vosseleri*. Insectary breeding. Continuous oviposition by 70 females.
A. Production of 1st-instar nymphs. Two-day totals.
B. Production of adults. Two-day totals.

103.5, which, from 70 females, represented 1.5 nymphs per female per day. In spite of the provision of a continuous supply of grains suitable for oviposition and the maintenance of the female population at a constant level, the emergence of nymphs has been variable with fairly well-defined peaks of emergence at intervals of 10–14 days. Whether this represents a true rhythm of oviposition or is an artefact associated with the method of breeding has yet to be determined.

(b) *Variation in nymph-production.*

Examination of the production of nymphs from individual batches of grain showed wide and irregular variations in total emergences. Two factors appear to have been mainly concerned. As has already been mentioned, fungal growth on the grains while these are in the hatching cages is a major factor in preventing the emergence of nymphs. It is probable that the conditions in these cages can be improved. There are, however, two conflicting requirements; on the one hand, the humidity must be kept sufficiently high to reduce drying of the grains to a minimum, and on the other, it must be kept down to reduce the growth of fungi.

The sudden drop in nymph-production during March was due to the second factor, *viz.*, the use of grains which were too old. This has been discussed in an earlier section; the occurrence is mentioned here to emphasise the paramount importance of selecting grain of the right age. Reintroduction of the younger grains in this instance immediately restored nymph-production.

(c) *Production of adults.*

In fig. 2, where the production of first-instar nymphs and adults are shown on the same scale, it is evident that the survival of nymphs is poor. During the 100-day period from 2nd January to 10th April, 5,022 first-instar nymphs emerged; of these, only 2,141 or 42.6 per cent. matured to adults. The percentage from successive batches has varied from 27.4 to 65.0. It will be shown in a later section that the first instar appears to be the most vulnerable stage, and the handling that is unavoidable in the course of counting may throughout have contributed to the high mortality.

The production of the 2,141 adults required 283 females and 220 males, of which 66 females and 51 males were still alive at the end of the 100-day period. The increase in the population was therefore rather more than five-fold.

Life-history.

The data presented here were obtained during the development of the breeding method, when all observations were made in an open, shaded insectary. The average maximum and minimum temperatures during this period were 78°F. and 64°F., respectively.

Incubation period and hatching.—The incubation period was determined on individual eggs laid in the hypocotyls and cotyledonary petioles of cotton seedlings. It varied from 7 to 10 days, with a mean value of 8.4 days.

The process of hatching was observed on several occasions. During development, the embryo appears to increase in volume, presumably by absorbing water from the surrounding plant tissue. Thus, 48 hours before hatching, the egg-cap, which is originally level with or slightly below the plant surface, is raised above the surface. It is attached to the body of the egg by a ring of fibrillae, and when hatching commences the fibrillae break, the cap is pushed aside, and the larva wriggles actively until all but its posterior tip protrudes from the egg. In this stage the nymph is completely enclosed within a "larval" skin, and as Gwynn surmised, could be described as vermiform. This skin splits dorsally, and the nymph continues to wriggle and rapidly frees itself; as soon as the legs are extended it walks up the plant, leaving the collapsed larval skin attached to the mouth of the empty egg-case. The whole process of hatching was always completed in less than three minutes, the majority of nymphs emerging between 2 and 4 p.m., when air temperatures were at a maximum.

Nymphal development.—Using young sorghum grains as food, individual nymphs were reared in glass tubes (4 × 1 in.) plugged with cotton wool. The food was changed every two days, and the duration of each instar and mortality at each stage were noted. The fates of 100 first-instar nymphs are shown in Table II.

TABLE II.
Nymphal development and mortality.

Instar	1	2	3	4	5	Adult
Total started	100	67	62	59	57	56
Died	33	5	3	2	1	
Per cent. loss	33.0	7.5	4.8	3.4	1.8	
Range of duration in days	3-5	2-4	1-4	2-4	3-6	
Average duration in days	3.7	2.6	2.4	3.1	4.9	

The highest mortality occurred in the first-instar nymphs. These are extremely active, and commence feeding within a few minutes of emerging. They are also very fragile and difficult to handle, and the high mortality occurring in this stage may have been largely due to the handling involved in transferring them to the tubes. There were indications, however, that this stage requires an atmosphere almost saturated with water-vapour for optimal survival. By providing the tubes containing first-instar nymphs with a slip of moist blotting paper, the mortality was reduced from more than 50 to 33 per cent., which includes individuals drowned in condensed moisture. The overall percentage of nymphs maturing to adults was 56.

The length of nymphal development averaged 16.7 days, the instars of greatest duration being the first and fifth. The periods of development of males and females were approximately equal, and the sex ratio approached unity. The range of development is shown in Table III.

TABLE III.
Nymphal development.

Total period as nymph (days)	Number of individuals		
	Male	Female	Total
14	2	0	2
16	11	14	25
17	8	8	16
18	5	6	11
19	0	1	1
22	0	1	1
Total	26	30	56
Average (days) ...	16.5	16.9	16.7

Longevity of females kept in captivity.

Females that had been reared on sorghum in tubes were kept singly in oviposition cages. Each was provided with a male and supplied with 100 milky sorghum grains which were changed every four days. The lengths of life and the total numbers of nymphs produced were recorded. Owing to a shortage of cages, only a few females could be studied; survival was unexpectedly long, ranging from 17 to more than 57 days, and the average length of life of six that died was 42.6 days.

The method employed did not enable a record to be made of individual total egg-production. Fluctuations in nymphal emergences were observed, but these may have been associated with variations in the proportions of eggs hatching from the grains. In contrast to the emergence of nymphs in the large-scale breeding, there seemed to be no definite pattern of emergence in this instance. Individual fecundity and the pattern of oviposition under more constant environmental conditions require further study.

The maximum number of nymphs produced by any one individual was 139, over a period of 47 days, an average production of 2.95 per day. The production of nymphs per day during each female's life-time ranged from 0.4 to 2.95. The maximum production so far achieved in the larger-scale breeding, *i.e.*, 1.5 nymphs per female per day, seems therefore to be near the average of what could be expected under these conditions.

Improvements towards Mass-rearing.

The average daily production of adults during the 100-day period referred to earlier was 21. The daily requirement of adults for maintaining the breeding was 2 males and 3 females. This meant that a little less than 25 per cent. of the adults produced were required for maintaining the breeding, leaving the remaining 75 per cent. for experimental purposes. Knowing the production of first-instar nymphs and the percentage survival to adults, it was possible to decide fairly accurately what numbers of nymphs and adults would be available for experimentation.

The production of these has not, however, been sufficient for carrying out any detailed and exhaustive experiments involving, for instance, comparison of development on different host-plants, or reactions of different cotton varieties. In order to meet these requirements, and also the demands of a specialist in insecticides, a minimum daily production of 200 adults would be required. Increasing the scale of breeding to this mass-rearing standard could be achieved by maintaining a larger number of females for egg-production and improving the efficiency of the breeding.

The two most important factors being studied for improving the efficiency are increasing the percentage of eggs hatching, which mainly involves eliminating the growth of fungi during incubation, and reducing the mortality of the nymphs. At the same time, cages have been constructed for maintaining 200 females, in groups of 25, for oviposition. These cages are illustrated in Plate VII, fig. 2. Their overall external measurements are $9 \times 9 \times 18$ ins., and they are divided by a vertically sliding partition. The front has two doors and the back two glass windows, and the top and sides are covered with "Tygan", a nylon gauze, of mesh 24×18 . The twin-unit design was selected mainly because it enables the sorghum to be changed without undue disturbance of the adults. Each half of the cage can accommodate a shallow, rectangular, metal tray, half-filled with vermiculite which is covered with plaster of paris, having holes in which are inserted the stems of clusters of sorghum grains, as in the smaller, circular cages. One half of each cage is set up with adults and sorghum grains. When the grains are due to be changed, fresh ones are inserted into a second tray and placed in the other half of the cage. By raising the sliding partition, and agitating the old sorghum, the adults are induced to move across to the new grain; they do so more readily towards the light from a window. When this transference is complete, the partition is lowered, and the old grains are set aside for the eggs to hatch. At the same time any dead adults are examined, and the requisite numbers of males and females replaced.

In the hatching cages, the grains containing eggs are placed with alternate rows of fresh grain which is introduced when the eggs are due to hatch. With this arrangement there is no need to handle the nymphs, for on hatching they quickly find the fresh grains and proceed to feed on them. When sufficient time has elapsed to allow all the eggs to hatch, the old grains are removed, and the process of supplying fresh grain for rearing the nymphs can begin. With this arrangement, it is confidently expected that the breeding will be sustained at a sufficiently high level to meet all experimental demands.

Production of a continuous Supply of Sorghum in the Field.

The method of breeding depends entirely on the provision of a continuous and adequate supply of young sorghum grains, which can only be achieved by a succession of sowings in the field. For convenience, these have been grown in small plots on an easily accessible enclosure, and under local conditions the maximum interval possible between successive sowings to ensure a continuous supply of grains has proved to be 12 days. It is not surprising, therefore, that this in itself has led to difficulties in the form of insect pests.

The major insect pest so far encountered has been the sorghum midge, *Contarinia sorghicola* (Coq.), which had not previously been recorded from Uganda and first appeared in September, 1951, in the original sowing of a wide range of varieties. If left uncontrolled the midge population rapidly builds up under these conditions, and eventually causes complete sterility in all of the heads, which are attacked as soon as they emerge from the sheath. Owing to the fact that the grains are required for the breeding of *L. vosseleri* only 16 days after the emergence of the heads, the use of any residual insecticide is precluded. The most satisfactory method of control, and one which is feasible on small plots, is to cover the heads with bags

as soon as they emerge, and to leave these on until the 16th day, when the grains are ready for use. The procedure has two further advantages, in that it provides protection from birds and keeps individual varieties free from cross-pollination.

The second type of pest is a complex of several Anthomyiid flies, including a species of *Atherigona*. The larvae of these kill the central portion of young shoots ranging in length from 6 to 18 ins. The main effect of these pests is to delay flowering, thus causing a serious dislocation in the production of a steady supply of young grains. The damage, however, results in the production of extra tillers, which to a considerable extent offsets the loss of damaged shoots. The attack occurs at a very early stage in the plants' growth, so that even residual insecticides can be used without incurring the likelihood of contaminating the heads. Control in this instance is being effected by the use of a dust containing a mixture of DDT and BHC, applied at the bases of the young plants where the eggs are laid, and on the adjacent ground. The first applications are made shortly after germination, and thereafter treatment is at weekly intervals until the plants are 2 ft. high.

Reference has already been made to the growing of a wide range of sorghum varieties in order to select one or more for the particular purpose of breeding *Lygus* in the laboratory. Experience has now shown that the characters best suited to this purpose are: (a) *dwarf habit*, for convenience of handling; (b) *early maturity*, which enables a succession of flowerings to be established rapidly; (c) *open panicles*, a very important character under local conditions, to minimise mould in the young heads; (d) *long seed-stems*, for inserting into the rearing cages; and (e) *large grains* which result in less interference of feeding with oviposition, and also contain more moisture for eggs to absorb. Out of the 82 varieties originally grown, six were selected, each possessing some of these characters, but none having all. The breeding has been carried out on two of these selections, which combine all characters except (e). It is because sorghum varieties may differ in their suitability for *Lygus*-breeding that it is desirable to standardise the breeding on one variety. With the co-operation of a plant-breeder specialising in sorghum selection, it is hoped to produce one variety containing all of the above characters.

A search for a sorghum unsuited to *Lygus*-breeding might be a fruitful line of investigation, and might lead to some degree of field control in some areas. This type of resistance in sorghum has already been indicated where *Dysdercus superstitionis* (F.) is concerned (Geering, 1952).

Eleusine coracana was mentioned earlier as a possible medium for breeding *Lygus* in captivity. Although *L. vosseleri* breeds profusely on this crop in the Eastern Province of Uganda, it is less suited to artificial breeding than sorghum, chiefly because the grains, being small, dry out rapidly on being cut from the plant. In addition, *Eleusine* has not grown satisfactorily in the climatic conditions of Namulonge, a part of Uganda where it is not normally grown as a crop. Sorghum was therefore chosen in preference.

Application of the Technique to other Mirids.

Employing exactly the same procedure as for *L. vosseleri*, this method has been used successfully for breeding *Megacoelum apicale* Reut. in the laboratory. This is a very much larger Mirid, which in the field also breeds on sorghum and cotton; its exact status as a pest of cotton is not yet known. A second species of *Lygus*, *L. virens* Taylor, which enters cotton fields and causes some damage, also breeds profusely on sorghum in the field. A study of this particular species, which apparently is unable to breed on cotton (Taylor, 1947), may provide useful information in solving the problem of controlling *vosseleri*. There is every reason to expect that it too can be satisfactorily bred by this method.

Conclusion.

At the outset, the aim of the work described here was to develop as quickly as possible a method for breeding continuous supplies of *L. vosseleri*. This has been achieved, but there is still room for improvement in the method. Such improvement will partly depend on a closer study of the biology and physiology of the insect. These studies, which can now be coupled with a critical examination of the nature of *Lygus* damage to cotton and with an intensive search for resistance, may in time provide clues to the control of this pest.

Summary.

Methods have been developed for breeding continuous supplies of *Lygus vosseleri* in the laboratory. Adults readily oviposit in cotton seedlings, but tend to damage the tissues by feeding, so that the eggs fail to hatch. Portions of developing sorghum heads were found more satisfactory; grains in the milk stage stay fresh long enough for eggs laid in them to hatch, if the ratio of adults to grains is kept low.

The method used was to expose sorghum grains to adult *Lygus* for 3-4 days, afterwards removing the grains to hatching cages to await emergence of nymphs. On emergence, nymphs were transferred to rearing cages and fed immature sorghum grains, changed every two days. Hatching and rearing cages consisted of muslin-topped, celluloid cylinders fitting on to shallow tins containing moist vermiculite covered by plaster of paris in which small holes received the sorghum stems.

At insectary temperatures the incubation period averaged 8.4 and the nymphal life 16.7 days; adult females survived up to 57 days and production of nymphs per female per day ranged up to 2.95. The mortality in the egg stage is not yet known: the growth of fungi on the sorghum grains during incubation causes losses. Mortality in the nymphal stages averaged 44 per cent. and occurred chiefly in the first stage, probably due to handling. Improvements to reduce losses are described.

Difficulties in maintaining continuous supplies of sorghum grains, caused by attacks of *Atherigona* on sorghum seedlings and of *Contarinia* on sorghum heads, can be overcome, respectively, by the use of insecticides and by bagging the emerging heads.

Acknowledgements.

The author wishes to express his indebtedness to Mr. E. O. Pearson for continued advice during the course of this work, and to Mr. A. P. G. Michelmore, Senior Entomologist, Uganda Government, for access to unpublished records, and permission to quote from these. Thanks are also due to Dr. J. B. Hutchinson and other colleagues for reading and criticising the manuscript.

References.

- GEERING, Q. A. (1952). A cotton stainer (*Dysdercus supersticiosus* Fabr.) as a potential pest of sorghum.—Emp. J. exp. Agric., **20**, p. 234.
- GWYNN, A. M. (1940). *Lygus* spp. In Tothill, J. D. Ed. Agriculture in Uganda, pp. 234-238. London, Oxford Univ. Press.
- HANCOCK, G. L. R. (1935). Notes on *Lygus simonyi*, Reut. (Carpidae), a cotton pest in Uganda.—Bull. ent. Res., **26**, pp. 429-438.
- HARGREAVES, H. (1934). Report of the Government Entomologist for 1933.—Rep. Dep. Agric. Uganda, 1933, pt. 2, pp. 45-47.
- TAYLOR, T. H. C. (1945). *Lygus simonyi*, Reut., as a cotton pest in Uganda.—Bull. ent. Res., **36**, pp. 121-148.
- TAYLOR, T. H. C. (1947). On the identity of the Cotton Capsid of Uganda.—Bull. ent. Res., **37**, pp. 503-505.
- WIGGLESWORTH, V. B. (1944). The principles of insect physiology.—2nd edn., pp. 2-3. London, Methuen.



FIG. 1. Oviposition cage set up with young sorghum grains on left and hatching and rearing cage with cover removed on right.

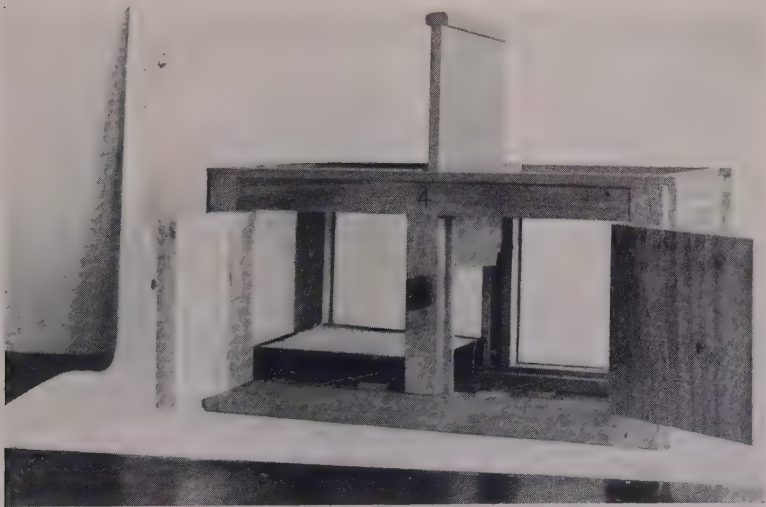


FIG. 2. Large oviposition cage for mass rearing.

THE SORGHUM MIDGE, *CONTARINIA SORGHICOLA* (COQ.), IN EAST AFRICA.

By Q. A. GEERING.

*Empire Cotton Growing Corporation, Cotton Research Station, Namulonge,
Uganda.*

In 1945, E. McC. Callan published a review of the world distribution of *Contarinia sorghicola* (Coq.). He showed that this insect had by then been recorded from each of the five continental areas, and the Pacific Islands. There was then, however, only one record in the literature of this insect's occurrence in Africa; this is Cowland's account in 1936 of his observations in the Anglo-Egyptian Sudan. The only paper relating to Africa which has appeared since then is one by A. Chiaromonte (1949) in which he reviews all the available information from Eritrea and Italian Somaliland, and concludes that the midge does not occur in those countries. A short account is given here of the first record of the midge attacking sorghum in Uganda, and reference is also made to its occurrence in some other African territories, where it has not previously been recorded.

In Uganda, the midge was recorded attacking sorghum in 1951, at the Cotton Research Station, Namulonge. This station is 16 miles north of Kampala and 20 miles from Lake Victoria, where the predominant vegetation is elephant grass. Sorghum is not grown for food, but many peasant holdings include small plots (up to half an acre) of dwarf sorghums used for brewing beer. These are planted in March, and are ratooned on being harvested in July, so that they flower again during September to October.

The conditions under which the midge was first discovered at Namulonge were as follows. A total of 82 sorghum varieties, obtained from Serere in the Eastern Province of Uganda, were sown (after fumigation) in single row plots. The varieties ranged in habit from dwarf, early types, to tall, late types, with all combinations of open, closed, and swan-necked panicles. They were sown in early July and the earliest varieties flowered during early September. The object in growing these varieties was to select a type suitable for breeding *Lygus vosseleri* Popp. in the laboratory. In mid-September, seed stems bearing young grains were placed in tubes for rearing *Lygus* nymphs, and it was from aborted spikelets on these seed stems that the first adults of *C. sorghicola* emerged. The infestation in the early varieties was negligible, and did not seriously affect the production of grains. As the later varieties came into flower, the attack increased until in the latest varieties, flowering six weeks after the earliest, it was estimated that 50 per cent. of the grains aborted as a result of midge attack. By that time, the early varieties had produced secondary tillers, and these were all completely sterilised by the midge. The adult midges begin ovipositing at the time the panicle emerges from the sheath and continue until all the stamens have withered, and in order to obtain adequate supplies of seed from the tillers all heads had to be protected by paper bags from the time of emergence.

Although no intensive study of the midge has yet been made, some information on its life-history under Uganda conditions has been accumulated. The presence of midge damage is first indicated by the occurrence of aborted spikelets, associated with a reddening of the glumes. If these are opened, one or more young white larvae are found close to the young aborted grain, under the pales. The larva feeds externally on the grain and assumes a bright orange colour when fully grown. Usually only one larva matures in each spikelet. After the midge emerges from the

pupa, the white empty pupal case can be found protruding from between the glumes of the sterile spikelet. The orange-bodied female midges can be observed actively ovipositing in the morning, between 9 and 11 o'clock, on fine days. On dull days they have been seen ovipositing continuously from 9 a.m. to 5 p.m. This is in marked contrast to the behaviour noted in the Sudan where, under very different climatic conditions, the midges are only active in the early morning (Cowland, 1936).

When a succession of sowings on small plots was initiated for the supply of grains for breeding *Lygus*, protection of the heads by bagging had to be adopted, and this has now been done continuously during the past ten months (January–October, 1952). The midge population has been greatly reduced in consequence. Throughout this period, however, some heads were left unprotected, and these were attacked continuously. Seed stems from these heads, collected 14–18 days after flowering, when larvae are fully grown, were placed in tubes in an open, shaded insectary and the emergence of adults was recorded. This showed that the population built up to a peak of 60 adult midges per 100 grains during early March. Thereafter, the infestation fell off and parasites became increasingly active; the percentage parasitism increased to 100 in larvae formed during early April. The life-cycle is rather longer than that recorded by Cowland (16 days) in the Sudan. By taking the time from the emergence of a panicle from the sheath (when the first eggs are laid) to the first emergence of adults from the same panicle, the period has ranged from 19 to 25 days.

Diapause larvae have been recorded in December, February, March and April. These all produced adults during early August, the duration of the diapause (under insectary conditions) plus that of the pupal period, for larvae formed in the different months, being as follows: December, 223–227; February, 180–188; April, 120–128 days. This suggests that some climatic change may have terminated the diapause of all larvae, irrespective of the time at which they were formed. The re-commencement of emergence occurred after a 20-day period of low saturation deficit of the air, and this was probably the factor concerned. Walter's results (1941) partially confirm this. He showed that, under natural conditions, there was a flush of emergence from diapause larvae in the field within 11 days following rain, at mean temperatures above 80°F.

In the Sudan, and America, it has been shown that the midge breeds in wild sorghum grasses, notably *S. halepense* and *S. sudanense* (Walter, 1941). These grasses do not occur in Uganda, but *S. verticilliflorum* is widespread in the bush and is a common fallow weed. Midges morphologically indistinguishable from *C. sorghicola* have been bred from the latter, and also from *Eleusine coracana*, which has not previously been recorded as a host-plant. Dr. H. F. Barnes of Rothamsted Experimental Station, who identified the midges, pointed out that morphologically identical specimens reared from different species of plants may well be physiologically and genetically distinct. The relations between the wild and cultivated sorghums are so close (Evelyn, 1951) that genetic differentiation among midges that feed on them seems unlikely, but an assessment of the status of the midge breeding on *Eleusine coracana* must be postponed until cross-feeding experiments have been conducted.

The parasites associated with the midge are species of *Tetrastichus*, *Aprostocetus* and *Eupelmus*. Species of these genera have been recorded as parasites of the midge in America, where it was probably introduced in imported seeds during the latter part of the last century (Walter, 1941), and also in the West Indies (Callan, 1941). In Uganda, the dominant parasites are *Tetrastichus* sp. and *Aprostocetus* sp.

The discovery of the midge in Uganda led the writer to institute enquiries concerning its occurrence in other East African territories. Mr. H. Doggett of the

Tanganyika Department of Agriculture is engaged on sorghum-selection work at Ukiriguru in the Lake Province of Tanganyika. When visiting Namulonge, he was able to see the adult midge in the field and the results of its attack. He has now reported that it was active at Ukiriguru during April, 1952. The writer has also observed it breeding on ratoon sorghum in late September throughout the whole of the Nyanza Province of Kenya. S. H. Evelyn has recently reported that he observed it in the field at Chitala (Domira Bay) in Nyasaland in 1950. It would be a mistake to conclude that the midge has recently spread from Northern Africa to these other regions. There is apparently substantial evidence (Evelyn, 1951) in support of the theory that the cultivated sorghums originated at least 2,000 years ago from wild types in the Kordofan Province of the Sudan, and that they have since spread throughout Africa and the world. It is in this part of Africa that the midge is an established pest and it is reasonable to suppose that it may have been disseminated at an early date with the spread of the sorghum. The position in Eritrea, however, as reported by Chiaramonte (1949), is unexpected, since this area is adjacent to Kassala Province of the Anglo-Egyptian Sudan, where the insect is abundant. It is worth noting in this connection that R. R. Anson (1952) reports that it is also an established pest in the Abyan area of the Aden Protectorate, and that control measures to minimise the losses it causes are part of the agricultural practice of the local Arab tribes.

It may well be that the midge is endemic in all areas where wild species of *Sorghum* grow, since it is probable that it evolved initially on these. This can only be confirmed by careful searching. It is known, however, that breeding occurs on three wild species of sorghum, *viz.*, *S. sudanense*, *S. halepense*, and *S. verticilliflorum*, all native to Africa. It is therefore to be expected that the midge occurs in all of the main sorghum-growing areas of Africa, and that the absence of records can be ascribed to the fact that in few areas does it do any noticeable damage, so that it has generally been overlooked by entomologists preoccupied with studies of other pests of known economic importance.* With schemes in progress for the increase of sorghum-production in established areas, and for its introduction into new areas of Africa, the people concerned, especially plant-breeders, would be well advised to watch for the midge. It can cause serious interference, as at Namulonge, in breeding plots where varieties may flower in succession over a long period.

The question of resistance has been considered by several workers, but there is at present no published evidence of the existence of true resistance. Both Cowland and Walter, who studied the attack on different varieties, concluded that no varietal resistance existed in the types they examined, yet both recommended that non-tillering varieties with a short flowering period should be grown, as these suffered less than the opposite types. In correspondence, S. H. Evelyn has provided the following brief account of his observations at the Gezira Research Station in the Sudan. "I recorded midge attack annually in my observation plots, which usually carried many hundreds of varieties. Single rows of these varieties were sown in groups according to height and maturation period . . . I came to the conclusion that freedom from midge was quite definitely a genetic resistance as in any one group of, say, Dwarf varieties of 3'-4' high, all maturing in 80-90 days, some were very heavily attacked and others showed no attack, or only a very small percentage of empty glumes. The same observation held for Mediums, Talls, and Very Talls of a series of maturation groups. It did not seem that this could be only a 'preference' effect, although, of course, this cannot be completely ruled out." If this is, in fact, evidence of resistance, one factor which might be involved is the

*Specimens of *C. sorghicola* have recently been received at the Commonwealth Institute of Entomology from the Gambia and Nigeria and also a report that this pest is causing serious damage to sorghum in the Gold Coast.—Ed.

degree of apposition of the glumes, making it more or less difficult for the females to insert their ovipositors into the flowering spikelets.

Conditions in this part of Uganda are exceptional in that they permit the midge to breed throughout the year. In other areas, possessing a different climate, breeding would probably not be continuous (even if a continuous supply of hosts were made available), particularly where a prolonged dry season occurs. The extreme of these conditions is illustrated by the Sudan, where breeding is restricted to the rainy season (September to December), the long dry season being passed as a diapausing larva. Conditions in the moister parts of Uganda appear favourable for introducing sorghum as a food crop, growing it in the first of the two rainy seasons (March-May). One cannot at present say whether the practice of ratooning the beer sorghums would have any effect on such a crop, but it might result in a heavier midge attack than would otherwise be expected.

Summary.

Contarinia sorghicola was discovered in Uganda in 1951, the only previous African record being from the Sudan. The infestation started in September in early varieties of sorghum grown in observation plots and reached a peak on late varieties and ratoons in early March, thereafter declining as parasitism, chiefly by *Tetrastichus* sp. and *Aprostocetus* sp., increased to reach 100 per cent. in April.

The life-cycle was normally 19-25 days, but between December and April diapause larvae were found; these all produced adults in early August, following a period of high humidity.

Midges morphologically indistinguishable from *C. sorghicola* have been bred from *Eleusine coracana* and from wild *Sorghum verticilliflorum* in Uganda, and the midge may well be endemic wherever wild sorghums grow. Enquiries show that midge damage to sorghum occurs in Kenya, Tanganyika and Nyasaland, and it is likely that all the main sorghum growing areas of Africa will prove to be infested.

The possible occurrence of resistance is discussed.

Acknowledgements.

The writer would like to thank Dr. W. J. Hall, C.M.G., M.C., Director of the Commonwealth Institute of Entomology, for arranging identifications of the midges and associated parasites, and particularly Dr. H. F. Barnes for actually making the determinations of the midges.

References.

- ANSON, R. R. (1952). Aden Protectorate. The Abyan Scheme and Cotton Experiment Station, season 1951-52.—Progr. Rep. Exp. Stas Emp. Cott. Gr. Corp., Aden 1951-52, 5 pp.
- CALLAN, E. McC. (1941). The gall midges (Diptera, Cecidomyiidae) of economic importance in the West Indies.—Trop. Agriculture, **18**, pp. 117-127.
- CALLAN, E. McC. (1945). Distribution of the Sorghum Midge.—J. econ. Ent., **38**, pp. 719-720.
- CHIAROMONTE, A. (1949). Precisazioni su *Contarinia sorghicola* Coq. nell'Africa orientale italiana.—Riv. Agric. sub trop., **43**, pp. 195-198.
- COWLAND, J. W. (1936). The Sorghum Midge in the Anglo-Egyptian Sudan.—Ann. appl. Biol., **23**, pp. 110-113.
- EVELYN, S. H. (1951). Sorghum breeding in the Sudan.—World Crops, **3**, pp. 65-68.
- WALTER, E. V. (1941). The biology and control of the Sorghum Midge.—Tech. Bull. U.S. Dep. Agric., no. 778, 26 pp.

A CONTROLLED HUMIDIFIER FOR INSECT BREEDING ROOMS.

By W. H. KITCHEN and D. GALL.

West African Institute for Trypanosomiasis Research, Kaduna, Nigeria.

In a climate where very low humidities are seasonally associated with relatively low temperatures, the drawing of air over a wet screen will raise the humidity of a breeding room, but the moist air from the screen will often be so cold that it may lower the temperature of the room below the requirements of the insects ; it therefore becomes difficult to maintain both a high humidity and a high temperature at the same time. The particular problem was how to maintain a high humidity in a large tsetse-fly breeding room.

To overcome the difficulty referred to a simple atomizer was built and used as a humidifier, spraying the room with a fine water mist. This arrangement effectively fed a large volume of water into the air with negligible cooling.

The Apparatus.

The apparatus is illustrated diagrammatically in fig. 1. The atomizer head consists of two nozzles, drawn from 7 mm. glass tubing, set at right angles to each other. One tube is fed with compressed air at about 15 lb. per sq. in. from an Edwards type IV vacuum pump and compressor. The other leads to a reservoir of water. This reservoir should be kept as close to the atomizer head as possible since the weight of a long water column impedes a free flow from the nozzle. The water consumption varies between 1 and 5 litres per hour of continuous running, according to the design of the nozzles ; the most effective size of nozzle, and their relative positions, were determined by trial and error. Directing the spray towards the ceiling avoids saturating cages and other apparatus with water droplets.

To control the humidifier a hydrostat, based on the Friez humidostat (Peterson, 1949), and a relay, were incorporated into the compressor motor circuit. The hygrostat uses two 5 in. strands, each of 10 human hairs, as humidity detectors. Contraction of the hairs with falling humidity closes the left-hand pair of contacts ; a Sunvic, type F102-3M, hotwire vacuum switch provides a relay which switches on the compressor motor. The right-hand contacts are not in use, but if needed would serve as a means of closing a circuit on rise of humidity.

A breeding room 40 ft. long by 20 ft. wide is used of which some 20 ft. of the length is reserved for the breeding cages and is maintained at a relative humidity of about 80 per cent. at a mean temperature of 77°F. The remainder of the room is devoted to feeding, counting, distributing and recording, and here the humidity is allowed to fall slightly below the optimum. The atomizer has been sited in the breeding area, well above the cages, directed towards the rest of the room, the hygrostat being placed behind and below the atomizer among the cages. The hygrostat has been set by trial and error to maintain the optimum humidity of 80 per cent. ; it switches on at 80 per cent. relative humidity and off at 83 per cent. In practice, the relative humidity at the far end of the room remains about 5 per cent. lower than that in the breeding area.

A wet screen unit has been retained in the breeding room ; in the wet season the fans may be run with the screen dry, in which case the unit acts solely as a ventilator, but in hot weather, if run with the screen wet, it also provides a means of cooling the room at the same time of raising the humidity.

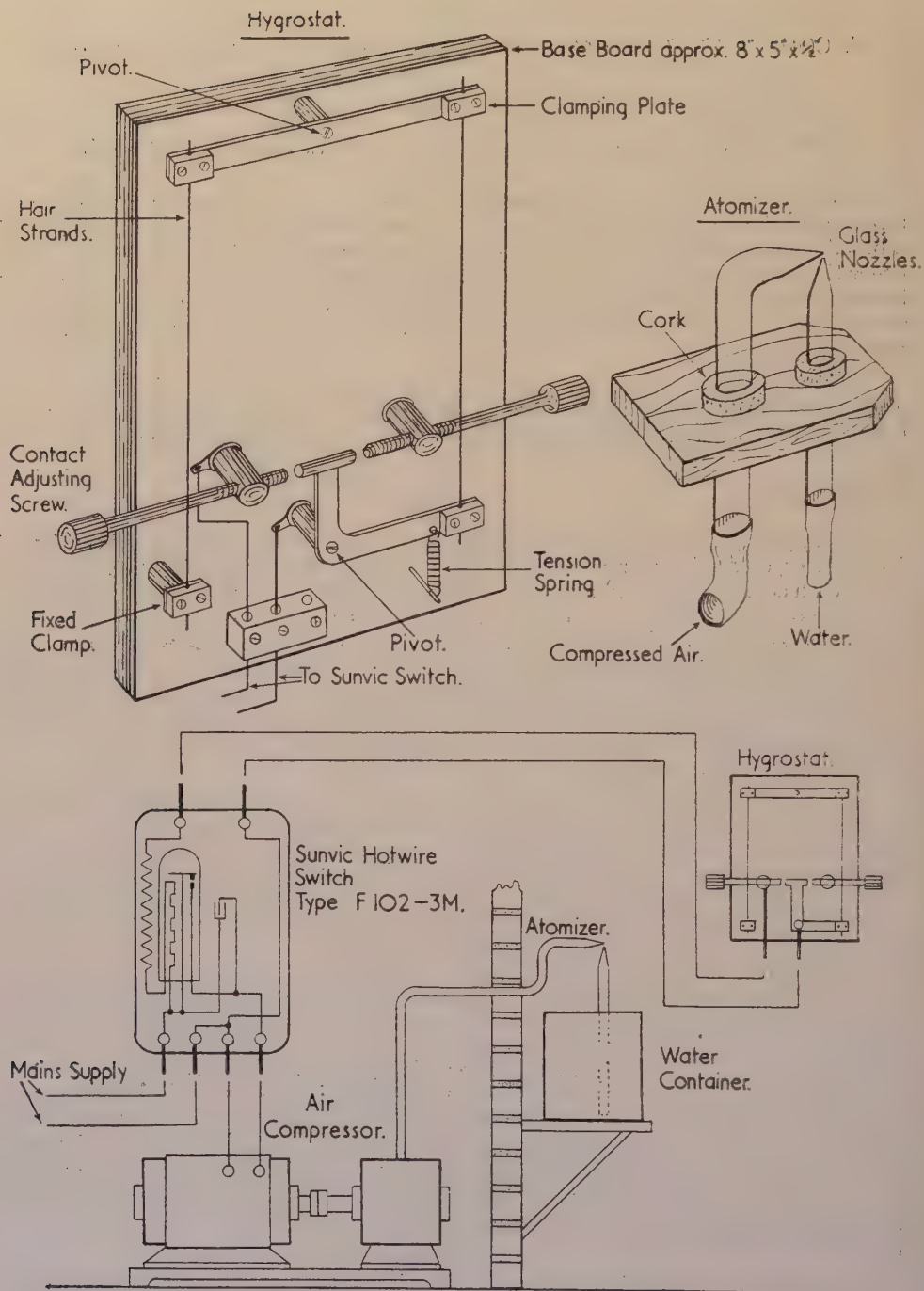


Fig. 1.—Diagram of the humidifier (not to scale), showing the atomizer head, the hygostat, and the general layout of the apparatus.

The atomizer as a humidifier has certain merits. It is compact and efficient. It is automatic in action and can be set within wide limits by the hygrostat adjusting screw, while the character and force of the spray can be altered by varying the size and setting of the atomizer nozzles. The spray can be easily directed wherever it is most convenient or effective. The apparatus does not need running water, and in practice requires very little attention beyond the daily filling of the reservoir.

Summary.

An atomizer, with controlling hygrostat, has proved to be a very satisfactory humidifier for raising the humidity of a large breeding room without lowering its temperature.

Acknowledgements.

We are indebted to Dr. T. A. M. Nash, O.B.E., Chief Entomologist, for much valuable advice, and to Colonel H. W. Mulligan, Director of the West African Institute for Trypanosomiasis Research, for permission to publish this paper.

References.

- PETERSON, A. (1949). A manual of entomological equipment and methods.—
6th edn. Ann. Arbor, Mich., Edwards Bros.
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THE POTENCY AND PERSISTENCE OF SOME NEW SYNTHETIC INSECTICIDES.

By J. R. BUSVINE, D.Sc., and Ruth NASH, B.Sc.

London School of Hygiene and Tropical Medicine.

LL.

This paper records the results of tests with certain pests of medical importance to measure the potency of some of the newer synthetic insecticides (*i.e.*, those more recent than DDT and BHC). The residual persistence of the compounds was investigated as well as their initial toxicity, these two qualities giving some idea of the potential value of a new insecticide, though several others are important in practice. In addition, data on the levels of susceptibility of various species, under standard conditions, may be of value for future reference should the development of resistant strains be suspected.

Samples and Test Insects.

The following insecticides were tested :—

DDT, recrystallised sample, M.P. 108°C. ; gamma BHC, recrystallised sample, M.P. 110°C. ; chlordane, technical grade (90 per cent.) ; dieldrin, technical grade (85 per cent. M.P. 153°C.) ; aldrin, technical grade (95 per cent. M.P. 90°C.) ; "Pyrolan" (1-phenyl-3-methyl-pyrazolyl-(5)-dimethyl carbamate (M.P. 50°C.)) ; toxaphene (65–70 per cent. chlorine content) ; allethrin, technical grade (80 per cent.) ; pyrethrins (25 per cent. concentrate).

Most of the samples were supplied through the Fungicides and Insecticides Research Co-ordination Service, the manufacturers being : Messrs. Geigy Co. (DDT & "Pyrolan"), Imperial Chemical Industries (BHC), Julius Hyman Inc. (chlordane, dieldrin, aldrin), Cocker Chemical Co. (toxaphene), U.S. Industrial Chemicals (allethrin) and Stafford Allen & Co. (pyrethrins).

The following Arthropods were used in the tests :—

Adults of : *Pediculus humanus* L., *Cimex lectularius* L. and *Aedes aegypti* (L.) (females only).

Nymphs of : *Rhodnius prolixus* Stål (4th, 5th stage).

Ornithodoros moubata (Murr.) (1st stage).

Data for adults of *Musca domestica* L. and *Xenopsylla cheopis* (Roths.) are quoted from other sources. A few curves were obtained for adults of *O. moubata* which were found to be considerably more resistant than the young nymphs, but not greatly different in relative resistance.

Some difficulty was experienced in obtaining a colony of *Pediculus*, but eventually some eggs were obtained from a laboratory culture in Cairo, through the kindness of Dr. S. Madwar. The lice of this strain were later shown to be abnormally resistant to DDT but apparently normally susceptible to other insecticides. Data for DDT in this paper were obtained with a non-resistant strain of lice obtained subsequently in London.

All species were reared at 27°C., except the lice, which were worn daily on the leg as described by Buxton (1947). The bugs, *Cimex* and *Rhodnius*, and the tick, *Ornithodoros*, were fed on the blood of a rabbit at intervals of 1, 3–4 and 4–6 weeks, respectively. The fleas and mosquitos were reared essentially as described by Leeson (1932). All the insects were tested in a well-fed condition, *Cimex* and *Ornithodoros*

3 days and 24 hours after a blood meal, respectively, while *Pediculus* was taken directly from rearing boxes and *Rhodnius* used up to 1 week after feeding.

Experimental Technique.

Principles involved.

The methods used were very simple, requiring no complex apparatus, except an incubator and a constant temperature room. Briefly, the insects were exposed to the insecticides by forcing them to walk for standard times on impregnated filter papers.

This general method was employed by Busvine and Barnes (1947) who used papers impregnated with acetone solutions, which gave dry deposits of the insecticides. Their results were not entirely satisfactory because, as they pointed out, the evaporation of a volatile solvent leads to irregular size and distribution of crystals, with consequent erratic results. Furthermore, a large change in deposit was required to affect the mortality of the exposed insects. Hadaway and Barlow (1951) have shown the importance of the size of crystals in dry residues of insecticides but, even with their carefully graded deposits from crystalline suspensions, there was no simple relation between dosage rate and mortality. Thus, with mosquitos exposed to residues on plaster blocks, they found no increase in mortality with DDT deposits over 3 mg. per sq. ft., which gave maximum effect.

For the measurements of insecticidal potency in the present investigation, the samples were first dissolved in refined mineral oil, at different concentrations, and the test filter papers were then impregnated with standard volumes of the solutions. The results of replicated tests were reasonably consistent and also changes in concentration were fairly sensitively reflected in changes in mortality of the exposed insects. The reason for the improved accuracy is that the papers were impregnated with a constant volume of solution, so that each type of insect would tend to be contaminated with the solvent to the same extent. The subsequent lethal effect, then, was directly related to the concentration.

Details of technique.

(a) Measurements of initial toxicity.

The insecticides were dissolved in Shell oil P: 31, a high-boiling refined paraffinic oil, and diluted to give a range of concentrations, at approximately equal logarithmic intervals, in the ratios 10 to 6 to 3 to 1.8 to 1.0, etc., according to the requirements of solubility and potency of the sample. Whatman No. 1 filter papers (11 cm. diameter) were then impregnated by wetting them as uniformly as possible with 1 ml. of a 1 : 2 mixture of oil solution and ether. The papers were hung up for one or two hours, by which time all the ether had evaporated and the non-volatile oil solution had spread evenly, leaving a residue of 3 mm.³ per sq. cm.

For most tests, the insects were confined on the treated filter papers under inverted glass funnels. The mosquitos, however, were enclosed in cylinders (2 × 1 in. diameter) made by rolling up the filterpapers and closing the ends with glass discs. In tests with gamma BHC, attempts were made to prevent undue concentration of the vapours. Thus, the crawling insects were confined in glass rings instead of funnels and the ends of the paper cylinders used for mosquitos were closed with mosquito netting instead of the glass discs.

All the exposures were made in an incubator at 30°C. for the following periods : *Cimex*, *Rhodnius*, *Ornithodoros*, 24 hours ; *Pediculus*, 18 hours ; *Xenopsylla* and *Aedes*, 1 hour. After exposure, the insects were removed to clean cages or tubes kept at 25°C. and inspected for mortality after the following periods : *Cimex*, *Rhodnius* and *Ornithodoros*, 7 days ; *Pediculus*, 2 days ; *Aedes*, 1 day ; *Xenopsylla*, 4 days.

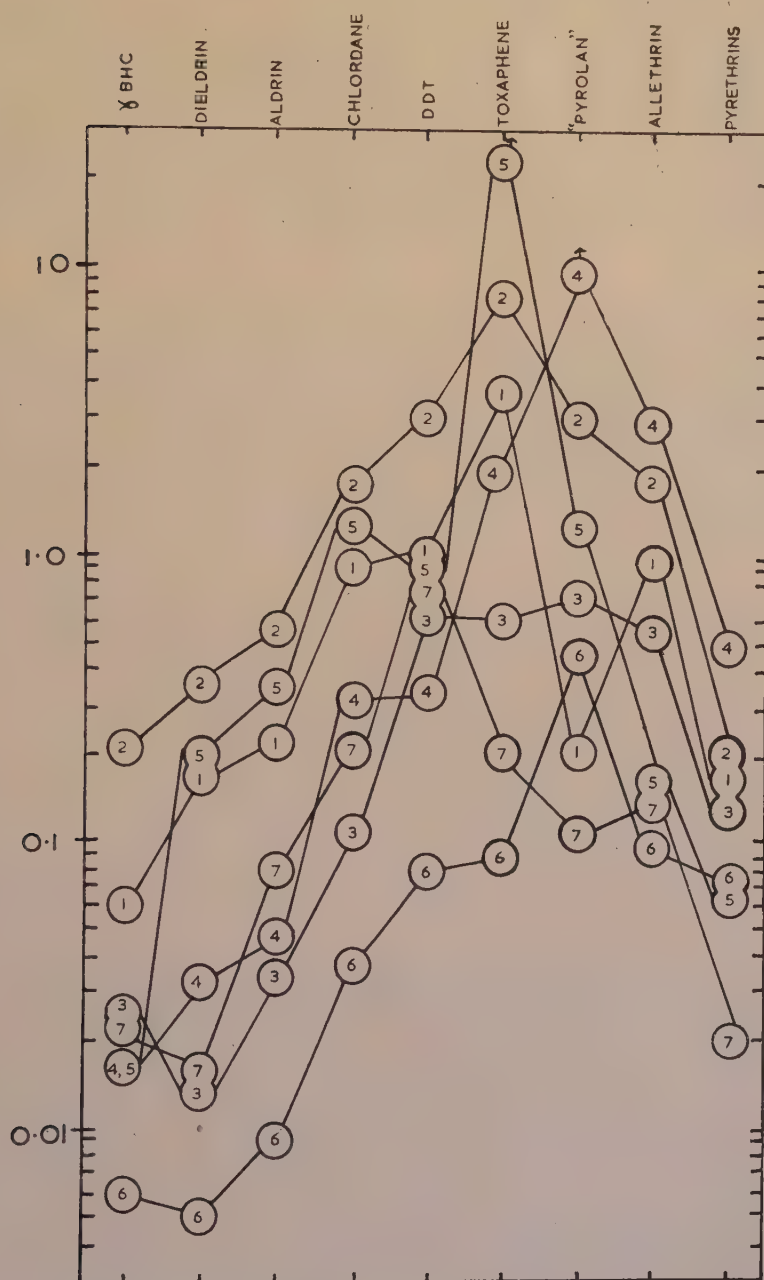


Fig. 1.—Median lethal concentrations (per cent.) of various insecticides to a range of medically important pests. The concentrations are indicated on a vertical logarithmic scale, and the various pests are indicated by numbers as follows:—

1. *Cimex*. 2. *Rhodnius*. 3. *Pediculus*. 4. *Xenopsylla*. 5. *Aedes*. 6. *Musca*.
7. *Ornithodoros*.

(b) Tests for residual persistence.

The duration of the residual action of the insecticides was assessed by mortality among batches of bed-bugs exposed to filter papers at intervals after treatment. Since accurate dosage-mortality curves were not required and it was not known what effect mineral oil might have on the persistence of the insecticides, these tests were made with dry residues from acetone solutions at two levels, 0.5 and 0.1 mg. insecticide per sq. cm. After treatment, the papers were hung up in diffuse daylight in a laboratory, the temperature of which averaged about 18°C. Tests were made at intervals of 1, 3 and 10 days and 1, 3 and 6 months.

Results.*Initial insecticidal potency.*

Only about a dozen insects were used in each batch, but tests at the critical concentrations were replicated 3 to 6 times. From the average mortalities, log-concentration/probit regression lines were drawn and the median lethal concentrations were estimated graphically. These figures are set out in Table I and illustrated by fig. 1, to which data for *Xenopsylla* and *Musca* are added for comparison. The figures for *Xenopsylla* are taken from a paper by Dr. A. A. Shawarby with his kind permission; those for *Musca* were obtained by a different method as described by Busvine (1951).

TABLE I.

Median lethal concentrations (per cent.) in the oil solutions of various insecticides to a range of medically important pests.

	γ BHC	Dieldrin	Aldrin	Chlordane	DDT	Toxophene	Pyrolan	Allethrin	Pyrethrins
<i>Cimex</i>06	.17	.23	.95	1.0	3.9	.21	.97	.17
<i>Rhodnius</i>21	.37	.58	1.8	3.0	8.0	3.0	1.9	.22
<i>Pediculus</i>025	.015	.038	.11	.60	.60	.74	.59	.14
<i>Xenopsylla</i>018	.032	.048	.33	.37	2.0	>10	2.9	.50
<i>Aedes</i>018	.20	.36	1.4	.95	(20)	1.4	.17	.065
<i>Musca</i>006	.005	.009	.036	.08	.09	.46	.097	.07
<i>Ornithodoros</i>024	.016	.080	.22	.75	.21	.11	.15	.02

Since the methods and periods of exposure for the different insects were not all identical, the data do not exactly indicate their relative resistance. On the other hand, the relative potencies of the different compounds may be directly compared and a general impression gained from fig. 1. It will be found that the order of effectiveness of the insecticides differs to some extent according to the kind of insect used for assay. As might be expected, this inconsistency is most marked between compounds of different types. Thus, there is good agreement in the order of potency of the allied compounds dieldrin, aldrin and chlordane and also between allethrin and pyrethrins; but there is little harmony between "Pyrolan" and either toxaphene or allethrin.

Considering the matter from the biological side, it might be thought that closely related species would react similarly, even to insecticides of different types. Our test insects are rather diverse, the most closely allied being *Rhodnius* and *Cimex*. These compare fairly well, the figures for the former being mostly about 2-3 times those for the latter; but there is a notable divergence for "Pyrolan" in which *Cimex* is 15 times as susceptible as *Rhodnius*.

Where there was evident inconsistency (indicated by resistance line sharply cutting across others in fig. 1) extra tests were made to confirm the relevant points.

These anomalous cases might be worthy of further study, to determine, if possible, whether the factor responsible was a physical matter affecting penetration or whether a biochemical difference was the cause.

Results of persistence tests.

The data given in Tables II and III must be interpreted with some caution, for the mortality of the bugs was affected by the intrinsic potency of each insecticide as well as by the amount of persisting. Nevertheless, where a decline in mortality is observed, there is evidence of failure of the insecticide to persist, either for physical or chemical reasons.

TABLE II.

Persistence of various insecticides shown by percentage kills of bed-bugs exposed, at different intervals after treatment, to filter papers impregnated at 0.1 mg. per sq.cm.

	3 days	10 days	1 month	3 months	6 months
Dieldrin ...	100	100	100	100	100
Toxaphene ...	100	100	100	100	100
DDT ...	100	100	100	100	100
Pyrolan ...	100	100	100	100	80
γ BHC ...	100	100	94	58	51
Chlordane...	100	90	65	50	26
Aldrin ...	100	95	54	50	23
Allethrin ...	100	100	87	24	12
Pyrethrins ...	9	0	—	—	—

TABLE III.

As Table II, but filter papers impregnated with 0.5 mg. per sq. cm.

	3 days	10 days	1 month	3 months	6 months
γ BHC ...	100	100	100	100	66
Chlordane...	100	100	98	92	59
Aldrin ...	100	100	95	68	51
Allethrin ...	100	100	93	80	41
Pyrethrins ...	87	32	4	0	—

The results of this series of tests place the insecticides in the following descending order of persistence.

1. DDT, dieldrin, toxaphene.
2. "Pyrolan".
3. gamma BHC, chlordane, aldrin.
4. Allethrin.
5. Pyrethrins.

This probably gives a fair idea of the persistence of residues of these insecticides, indoors in England.

Notes on certain samples.

Dieldrin is one of the most promising of the new insecticides, being highly effective and persistent.

"Pyrolan" appears to be of the same order of effectiveness as DDT. Against house-flies, dry films of "Pyrolan" were observed to have a fairly rapid paralysing effect, being about three times as rapid as the action of DDT; however, rather more

flies recovered from the "Pyrolan" later. This insecticide was found to be quite as toxic to two resistant strains of house-fly as to normally susceptible ones.

Allethrin was generally less effective than natural pyrethrins, the ratios of their potencies being: *Cimex* 0.17; *Rhodnius* 0.12; *Pediculus* 0.24; *Xenopsylla* 0.17; *Aedes* 0.38; *Musca* 0.72; *Ornithodoros* 0.13. The synthetic compound was relatively most effective against *Musca*.

The initial insecticidal action of toxaphene was generally lower than that of other compounds, but it showed a high order of persistence.

Summary.

Simple laboratory methods are described for assessing the potency and persistence of insecticides. Some of the newer synthetic insecticides have been tested by these methods against seven arthropod pests of medical importance.

Initial potency was judged by the mortality of insects exposed to standard films of oil solutions of the compounds, on filter papers. The approximate order of potency of the samples tested was:—

γ BHC > dieldrin > aldrin = pyrethrins > chlordane = DDT = "Pyrolan" = allethrin > toxaphene.

Residual action was judged by the mortality of batches of bed bugs exposed to films of the insecticides, at intervals up to six months after preparation. The approximate order of persistence was:—

dieldrin = DDT = toxaphene > "Pyrolan" > γ BHC = chlordane = aldrin > allethrin, pyrethrins.

Acknowledgements.

This work was undertaken in response to the observations of Professor P. A. Buxton, C.M.G., F.R.S., on the lack of information about the newer insecticides, in certain fields.

One of us (R.N.) has been in receipt of a grant from the Medical Research Council during the period of the investigation.

References.

- BUSVINE, J. R. (1951). *Nature*, **168**, pp. 193–195.
BUSVINE, J. R. & BARNES, S. (1947). *Bull. ent. Res.*, **38**, pp. 81–90.
BUXTON, P. A. (1947). *The Louse* . . .—2nd end., 164 pp. London, Arnold.
HADAWAY, A. B. & BARLOW, F. (1951). *Bull. ent. Res.*, **41**, pp. 603–622.
LEESON, H. S. (1932). *Bull. ent. Res.*, **23**, pp. 25–31.
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LABORATORY AND FIELD TRIALS IN THE CONTROL OF FLEAS AND LICE.

By A. A. SHAWARBY, D.T.M. & D.P.H.

Insect Control Section, Ministry of Public Health, Cairo.

There are many references in the literature to field trials in which DDT is used against human body lice and against fleas of the family PULICIDAE (Soper & others, 1947; Davis, 1947; Nicholson & others, 1948; Bushland & others, 1945; Kartman, 1946; Pollock, 1948; etc.). Published work on the use of BHC against the same insects, however, is very scanty. The available information in either case is more or less qualitative, and the evidence of effectiveness is mainly circumstantial, based on the control of epidemics rather than of insects. Quantitative data, e.g., the degree of lousiness or flea indices were only given in a few instances (Soper & others, 1945) in North Africa, and Machiavello and others (1946) in South America.

The effects of DDT and BHC against body lice and fleas in the field are compared quantitatively in the present paper. The laboratory experiments were conducted in an attempt to elucidate some of the field results.

Methods and Technique.

Laboratory Methods.

(a) Fleas.

Xenopsylla cheopis (Roths.) was bred according to Leeson's technique (1932). One- to three-day-old fleas were fed overnight on a white mouse at room temperature. For exposure to insecticide, filter papers were impregnated with 3 mg./sq. cm. of oil solution as described by Busvine and Nash (1953). The fleas were placed with 10 small (0.5 cm. diameter) discs cut from the treated filter paper in 5×1 in. flat-bottom tubes for one hour at 30°C. Thereafter they were transferred to clean tubes on a little sawdust and observed for 4 days at 24°C. and about 80 per cent. relative humidity. Control fleas were exposed in the same way to filter-papers treated with Shell oil P.31 only. For each concentration, 3-4 replicate experiments were made with an average number of 20 fleas each. The median lethal concentration of the insecticide was then found from probit-kill concentration curves.

Flea larvae were exposed in a similar manner to impregnated filter papers for one hour at 30°C. After exposure they were transferred to clean test tubes containing a little of the breeding medium and kept at 24°C. and 80 per cent. R.H., and the number dead counted after 48 hours.

Flea cocoons (4-9 days old) were tested in one of two ways, either by placing them on about 5 gr. of the breeding sand mixed with 2 per cent. by weight of the insecticidal dust or by spraying them in a Potter tower (Potter, 1941) with 2 cc. of the wettable suspension of the insecticide in water. They were thereafter kept at 24°C. and 80 per cent. R.H. until the controls emerged, or for 15 days. The cocoons were then opened and the contents inspected. Certain cocoons were empty though no fleas had emerged. This is due to disturbance of the larva, after it has made the cocoon: such cocoons were omitted, the percentage emergence being reckoned on the remainder.

Mud blocks 4×5 cm. and 1.5 cm. thick were prepared from earth obtained from a garden in the suburbs of London. They were allowed to dry at room temperature for about three days and were then sprayed in a Potter tower with 2 cc. of a water

suspension of insecticide. A deposit of 0.022 gm. DDT per sq. cm., as estimated by the Alessandrini chromatic scale method (Alessandrini, 1948), was left by treatment at this rate with a 0.5 per cent. DDT suspension. Adult fleas were exposed to the surface of the blocks (in an open tube 1.5 × 4 ins. fixed to the block by paraffin wax) for one hour at 30°C. and were kept under observation for 3 days, as in the filter paper technique.

(b) Lice (*Pediculus humanus corporis* Deg.).

The laboratory data for lice are taken from the preceding paper in this *Bulletin* by Busvine and Nash by their kind permission.

Field methods.

(a) Fleas (*Pulex irritans* L.).

The houses treated were typical Egyptian rural mud brick houses, in a village in one of the southern provinces of the Nile Delta. They were divided at random into 3 blocks of 96, 76 and 136 houses for BHC, DDT and control, respectively.

The floors and contiguous 2 ft. of wall surface of all the rooms and corridors were sprayed by means of the "Dobbins superbuilt" and "Dobbins power" sprayer of 10 litres and 20 gallons capacity, respectively, at a rate of about 40 cc. per sq. metre.

The flea population was measured at intervals by collecting adult fleas from the adhesive surface of a wooden board 15 × 25 cm. covered by a paper carrying a mixture of resin and castor oil. The trap was held by a handle and when knocked on its prominent edges on the floors, furniture, mats, etc., in every part of the house, the fleas disturbed jumped upon it and were caught. Results were compiled in such a way that the degree and intensity of infestation could be calculated. The great majority of the fleas collected in this way were *Pulex irritans* and only very few of the more than 10,000 examined were *Xenopsylla cheopis* or *Ctenocephalides* spp.

(b) Lice.

The clothes of the inhabitants of the above three groups of houses were dusted on the person with a plunger type hand-duster; each group was dusted with the insecticide used for spraying the same house.

The clothes of 573 people were treated with BHC, 436 with DDT and those of the remaining 551 with talc as a control group.

The infestation was estimated by examining at weekly intervals all the garments of each person and counting the lice they carried; the percentages of infested persons as well as the average number of lice per person were also found.

Insecticides.

For laboratory tests.

DDT	recrystallised sample	M.P. 108°C.
Gamma BHC	" "	" 110°C.
Chlordane	Technical Grade	(90 per cent.).
Dieldrin	" "	(85 per cent. M.P. 153°C.).
Aldrin	" "	(95 per cent. M.P. 90°C.).
Toxaphene	(65–70 per cent. chlorine content).	
Allethrin	Technical Grade	(80 per cent.).
Pyrethrins	(25 per cent. concentrate).	
DDT dispersible powder	50 per cent. of the 81 per cent. p,p' isomer.	
BHC	" "	50 per cent. of the crude BHC (13 per cent. γ isomer).

TABLE I.

Median lethal concentration (per cent. in constant weight of oil solution*) for *Xenopsylla cheopis* and *Pediculus humanus corporis*.**

Insecticide	gamma BHC	Dield.	Aldrin	Chlord.	DDT	Pyrethn.	Alleth.	Toxaph.
<i>Xenopsylla</i> (adults)	·018	·032	·048	·33	·37	·50	2·5	2·0
Times toxic as DDT	20	11	8	1·1	1·0	0·72	0·15	0·18
<i>Pediculus</i> ...	·025	·015	·038	·11	·60	·14	·59	·60
Times toxic as DDT	24	40	15·7	5·5	1·0	4·30	1·1	1·0

*Applied to filter papers at a rate of 3 mg. solution per sq. cm.

**Results for lice are quoted from Busvine and Nash (1953).

The diluent in the case of the two last mentioned was 40 per cent. china clay ; 10 per cent. Goulac was the wetting agent and both were kindly prepared by the General Chemical division of Messrs. Imperial Chemical Industries, Ltd., and subjected to the same milling treatment.

In field trials.

For spraying purposes the insecticides used were :—

(a) 50 per cent. wettable DDT powder (prepared by the Messrs. Cairo Socony Co. Ltd., chemical department, from 70 per cent. p, p' DDT so that 90 per cent. of the final powder passed a 250-in. mesh sieve and 10 per cent. a 300-in. mesh) used as a 5 per cent. water suspension to deposit a dose of 2 gr. DDT/sq. metre.

(b) 50 per cent. technical BHC dispersible powder (I.C.I. P 520) used as a 5·5 per cent. suspension in water to deposit a dose of 143 mg. gamma BHC per sq. metre.

For delousing purposes the dusts used were :—

(a) 10 per cent. DDT (70 per cent. p,p' isomer) in talc, with an average particle size of 5–17 microns.

(b) 3·08 per cent. technical BHC in diatomaceous earth (0·4 per cent. gamma BHC supplied by the I.C.I. in Cairo).

These mixtures were applied at an average rate of 40 gm. dust per person.

Results.

Laboratory.

The median lethal concentrations (M.L.C.) of some insecticides, obtained by the filter paper technique for *X. cheopis* and *P. humanus corporis* as the percentage of insecticide in oil solution applied at 3 mg. solution per sq. cm., are given in Table I. Their relative toxicity to fleas runs in the following descending order : gamma BHC, dieldrin, aldrin, chlordane, DDT, pyrethrins, allethrin, toxaphene. With lice on the other hand dieldrin heads the list while DDT ranks at the bottom with toxaphene.

On mud blocks the M.L.C. per cent. of DDT and BHC wettable powder sprays (when applied at a rate of 2 cc. of suspension in a Potter tower) for fleas (*X. cheopis* at one hour exposure) were respectively 0·028 and 0·038 per cent. corresponding to 0·023 pure p,p' DDT and 0·0085 per cent. pure gamma BHC showing that on this medium gamma BHC is a little less than 3 times as toxic as DDT. This great reduction of the comparative toxicity from 20 times on filter papers (see Table I) to only 3 on mud blocks might be accounted for—among other causes such as difference of surface and formulation—by a more rapid sorption of BHC than of DDT by the

mud blocks (Hadaway & Barlow, 1952b). This is illustrated by the following experiment: One hour exposure of fleas to deposits from 0.5 per cent. wettable suspension of either insecticide (corresponding to 22 mg. of p,p' DDT and 3.6 mg. of gamma BHC per sq. ft.) on mud blocks gave 100 per cent. kill one hour after treatment. Four days later the same blocks, kept at room temperature, gave 97 per cent. kill with DDT and only 11 per cent. with BHC. A 90 per cent. kill with BHC was obtained only after 24 hours exposure. These percentages are the average of 3 more or less consistent results from experiments with about a dozen fleas each. That the effect of BHC is to a great extent due to its fumigant action was shown by a 55 per cent. kill of fleas exposed for 24 hours on a platform that kept them away from direct contact with a block 4 days after treatment. Generally speaking these results parallel those obtained by Hadaway and Barlow (1952) with treated mud blocks. The comparatively low figures obtained with BHC are due, besides the different insects used, to the much smaller dose of gamma BHC applied (3.6 mg. against 25 mg. in Hadaway and Barlow's experiments).

The contact effect of BHC on the flea larvae appears to be even more toxic, as the M.L.C. obtained from exposure of small numbers of similar larvae to oil-impregnated filter papers is 0.005 per cent. against 0.18 per cent. for DDT, *i.e.*, gamma BHC is about 36 times more toxic to larvae than DDT.

On flea pupae both 10 per cent. DDT and 0.5 per cent. gamma BHC dusts completely suppressed emergence which was 80 per cent. in the control. The dose used was 2 per cent. by weight of the breeding medium equivalent to 0.2 per cent. of the pure DDT and 0.01 per cent. of the pure gamma BHC (p. 377). Similar results were obtained by sprays of 0.5 per cent. wettable suspension of either insecticide. Thus, unexpectedly, DDT caused a kill of the flea while still inside the cocoon. Further work is needed to find out the relative toxicity to pupae of the two insecticides.

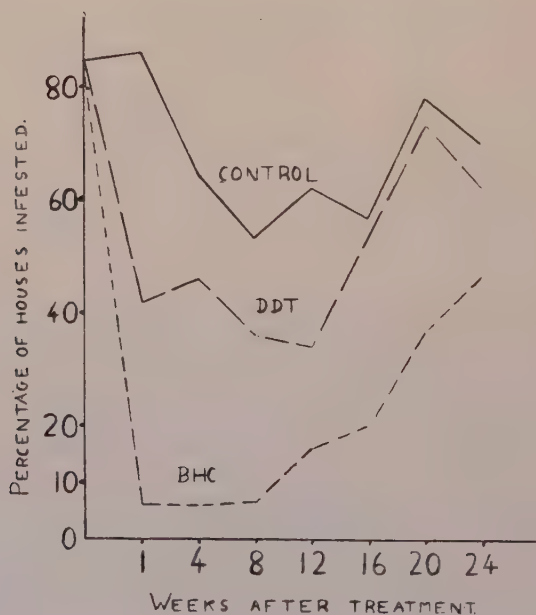


Fig. 1.—Percentage of house infestation with fleas.

*Field trials.**(a) Fleas (Pulex irritans).*

During April and the first half of May 1950, houses were searched for adult fleas three times before treatment with insecticides. During the third week of May the insecticides were applied. One week later the houses were searched again and from then onwards monthly collections were made until the end of November 1950. Results are shown in figs. 1 and 2.

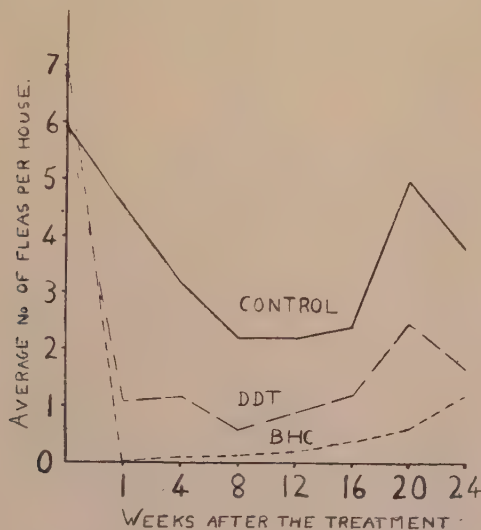


Fig. 2.—Average number of fleas per house.

It may be noted that the control group showed a drop of both the rate of infestation and the average number of fleas per house during the months of June, July and August (*i.e.*, 1 to 12 weeks after treatment) which is natural as the mean atmospheric temperature rises to about 30°C. and the relative humidity drops to about 45 per cent. (*P. irritans* is probably affected by atmospheric changes to a greater extent than *X. cheopis* which reacts more to the conditions of the rat burrow). This group in the case of the percentage houses infested remained at least for one week (after treatment of the other two groups) at a stationary high level (fig. 1). The treated groups on the other hand showed a much greater drop in both indices which was more marked in the case of BHC than DDT. They remained at the low levels for 8 and 12 weeks respectively after which they both rose more or less steeply. The effect of BHC was more pronounced than that of DDT, the initial reduction in the degree of infestation being 94 per cent. of its original value with BHC and only 55 per cent. with DDT. The average number of fleas per house, which is a more sensitive measure of the flea population, was still more responsive to BHC, which reduced it by 98.5 per cent. of the original value one week after treatment as against 78 per cent. for DDT. The infestation in houses treated with BHC remained at a much lower level than that of the control group 24 weeks after the application of the insecticide, but although this level was the highest for BHC since treatment, it was only twice that of the lowest for DDT attained only during July.

(b) Lice.

A field trial was conducted during the months of April to August 1950, inclusive. During the first six weeks 3 pre-treatment louse counts were made, and of 4,384 persons

examined 722 proved positive (16.4 per cent.). The number of lice counted was 5,375 (an average of 1.2 lice per person). The counts were continued at weekly intervals for 12 weeks after dusting. The results are shown in figs. 3 and 4. The last five weeks were excluded as the records were probably biased because :—

Many people refused to hand in their clothes and many of those who did, probably handed in unworn ones.

It was the time of the Fast of Ramadan when the people paid more attention to their cleanliness.

The efficiency of the counters was probably diminished through fasting.

On the whole during that period all groups showed very low counts but BHC had the lowest value of all.

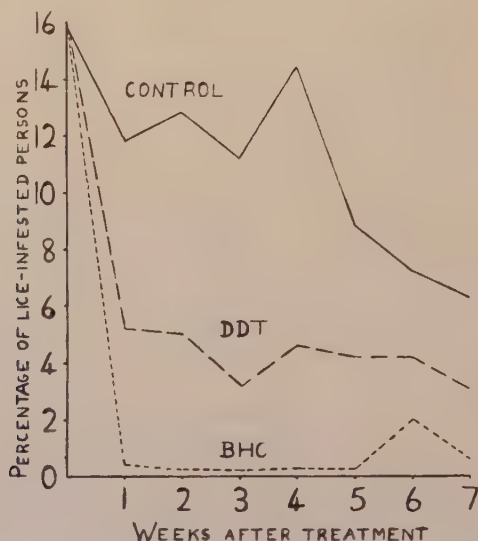


Fig. 3.—Percentage of persons infested with lice.

In the recorded seven weeks shown in the figures it may be noted that in the control group, after a preliminary drop of about 25 per cent., the percentage of infestation (fig. 3) remained at a more or less constant level for the next four weeks. In the case of DDT the percentage lousiness was reduced immediately by 73 per cent. of its original value (and remained approximately at that level) and the average lice per person (fig. 4) by 75 per cent., whereas BHC caused an immediate reduction of 97 and 99.5 per cent. respectively (a statistically significant difference). After that, the DDT-treated group tended to show an irregular rise in the average number of lice per person, which reached that of the controls by the sixth week; the louse population on the BHC-treated group remained at a very low level, though with slight fluctuations until the sixth week, when an appreciable increase took place. The percentage of infestation amongst treated persons remained more or less stationary up till the sixth week, when the BHC-treated group showed a rise. The latter measure is not expected to show a change until the surrounding community shows a heavy infestation which allows the lice to migrate from an infested to a clean person. In other words the first response of an increase in the louse population is seen in the average number of lice per person. Its rise in the insecticide-treated groups, with a tendency in the control group to drop, obviously means that the effect of the insecticide has started to wear off.

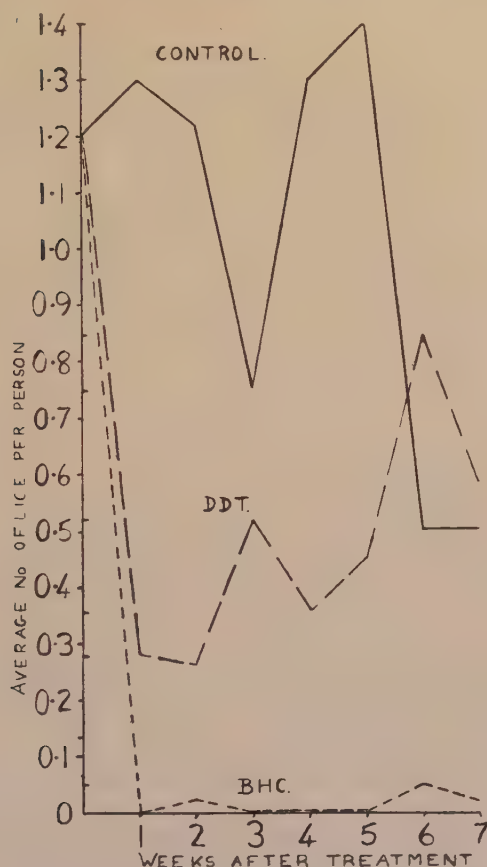


Fig. 4.—Average number of lice per person.

Discussion.

In the field trials with fleas, DDT and BHC gave an immediate reduction both in the percentage of infestation of houses and the average number of fleas per house, but BHC proved to be more effective than DDT. The degree of control of fleas which was achieved was maintained for 8 weeks with BHC and 12 weeks with DDT. The maintenance of this reduction was probably helped by atmospheric factors causing a reduction of flea breeding during June and July as evidenced by a similar, though very much smaller, drop occurring in the untreated group. After that period the number of fleas in both groups of treated houses began to increase. But since the level immediately after treatment was much lower with BHC, the level of infestation in this group remained lower than that in the DDT houses up to the end of the trial despite the somewhat longer persistence to be expected of DDT.

The more prolonged control obtained by BHC may be explained by:—

Gamma BHC is intrinsically 20 times more toxic than DDT; with the technique used in the present work, the M.L.C. in oil solution on filter paper for adult fleas is 0.018 per cent. for BHC as against 0.37 per cent. for DDT.

The contact effect of BHC on flea larvae appears to be even more toxic, gamma BHC being about 36 times more toxic than DDT.

On flea pupae, dusts containing 10 per cent. DDT, 0.5 per cent. gamma BHC and sprays of 0.5 per cent. wettable suspensions of either insecticide completely suppressed emergence.

Lastly the fumigant action of BHC probably favours a closer contact with the immature stages in the breeding medium and possibly also affects the adults while on the host.

Thus by immediate action BHC kills nearly 98.5 per cent. of the adults, while DDT kills only 78 per cent. and, by rapid sorption and fumigation effect, BHC is likely to come into early contact with the larvae to which it is much more toxic than DDT. In this way it is likely to effect a much greater reduction of the adult and larval populations which then take a longer time to build up. This is well illustrated by an average flea count, one week after treatment, of one adult flea per 10 houses with BHC against one flea per house in the case of DDT.

With body lice the effect of both insecticides broadly speaking runs parallel with that on fleas for the first six weeks, the period for which comparable figures are available, except that after DDT treatment, the louse population per person starts to rise much sooner than the flea population per house.

In conclusion, both insecticides are for all practical purposes equally efficient for the control of body lice or fleas; what BHC loses in persistence it gains in immediate effect. From the epidemiological point of view BHC would appear to be even more useful since the greater the reduction in numbers of insects below the critical level necessary for disease transmission the more rapidly and efficiently an epidemic could be controlled.

It remains only to discuss whether resistance of the insects to DDT (through routine and frequent use for delousing purposes) could be another contributing factor to the much greater reduction in population obtained by BHC treatment. Recent work on lice in Korea (Hurlbut & others, 1952) and Egypt (Busvine, 1953) gives an indication that in both countries strains have been evolved which possess a resistance to DDT. In the laboratory it was possible, by DDT selection, to get a strain of *X. cheopis* which, at the 4th generation, was fairly resistant to DDT; the M.L.C. for impregnated filter paper was 0.8 per cent. DDT or about twice the usual dose for susceptible fleas. It remains to get further proof in the field.

The possibility of the development of insecticide resistance calls for careful reconsideration of the wide programmes of routine use of insecticides followed in many parts of the world purely as a public health measure. If the insecticides are thus widely used, it may result in the vitiation of a valuable emergency measure.

Summary.

The median lethal concentrations of some persistent insecticides as obtained by a filter-paper-impregnation technique for *X. cheopis* and *P. humanus corporis* are given. Their relative toxicity runs in the following order:—

Flea (adults): Gamma BHC > dieldrin > aldrin > chlordane > DDT > pyrethrins > allethrin > toxaphene.

Lice: Dieldrin > gamma BHC > aldrin > chlordane > pyrethrins > allethrin > DDT=toxaphene.

On flea larvae gamma BHC is 36 times as toxic as p,p'DDT.

On flea pupae gamma BHC and DDT powders and wettable suspensions are effective; further tests are required to determine their comparative toxicity.

On mud blocks (London soil) gamma BHC wettable suspension is only 3 times as toxic as DDT compared with 20 times on filter papers impregnated with oil solutions.

This is probably due to the more rapid sorption of gamma BHC as well as the difference of surface and formulation.

In the field, at dosages of 2 gr. per m² of 70 per cent. p,p' DDT and 143 mg. of gamma BHC from wettable powders, BHC was superior to DDT as it gave a much better control of fleas (*P. irritans*) over the period of the experiment (24 weeks).

Field trials on body lice by dusting the clothes, while worn, with 40 gr. (per person) of dusts containing, respectively, 10 per cent. (70 per cent. p,p') DDT dust and 0.4 per cent. gamma BHC, showed that BHC gave more satisfactory results than DDT.

The resistance of lice to DDT shown by Busvine in the local strain of *Pediculus* and the possibility of its occurrence among fleas may be one of the factors contributing to a better control by BHC.

It was found possible to develop artificially, by selection in the laboratory, a slight degree of resistance in *X. cheopis*.

Acknowledgements.

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The field trials were made in Egypt, in the Insect Control Section, Ministry of Public Health, Cairo, under the direct supervision of Dr. S. Madwar and Dr. M. A. Shukry whose help and direction is very much appreciated. The participation of Mr. Y. A. Fatin and the junior staff of the section was indispensable.

References.

- ALESSANDRINI, M. E. (1948). Ann. Chim. appl., **38**, p. 53.
- BUSHLAND, R. C., McALISTER, jr., L. C., JONES, H. A. & CULPEPPER, G. H. (1945). J. econ. Ent., **38**, p. 210.
- BUSVINE, J. R. (1953). Nature, **171**, p. 118.
- BUSVINE, J. R. & NASH, R. (1953). Bull. ent. Res., **44**, p. 371.
- DAVIS, W. A. (1947). Amer. J. Hyg., **46**, p. 66.
- HADAWAY, A. B. & BARLOW, F. (1949). Bull. ent. Res., **40**, p. 323.
- HADAWAY, A. B. & BARLOW, F. (1952a). Trans. R. Soc. trop. Med. Hyg., **46**, p. 236.
- HADAWAY, A. B. & BARLOW, F. (1952b). Bull. ent. Res., **43**, p. 281.
- HURLBUT, H. S., ALTMAN, R. M. & NIBLEY, jr., C. Science, **115**, pp. 11-12.
- KARTMAN, L. (1946). Amer. J. trop. Med., **26**, p. 841.
- LEESON, H. S. (1932). Bull. ent. Res., **23**, p. 25.
- MACCHIAVELLO, A., MOSTAJO, B. & MOSTAJO NIJO, B. (1946). Bol. Ofic. sanit. panamer., **25**, p. 1097.
- NICHOLSON, H. P. & GAINES, T. B. (1948). Publ. Hlth Rep., **63**, p. 129.
- POLLOCK, J. S. M. (1948). Trans. R. Soc. trop. Med. Hyg., **41**, p. 647.
- POTTER, C. (1941). Ann. appl. Biol., **28**, p. 142.
- SOPER, F. L., DAVIS, W. A., MARKHAM, F. S. & RIEHL, L. A. (1947). Amer. J. Hyg., **45**, p. 305.
- SOPER, F. L., DAVIS, W. A., MARKHAM, F. S., RIEHL, L. A. & BUCK, P. (1945). Arch. Inst. Pasteur Algérie, **23**, p. 183.

INTER-RELATIONSHIPS OF THE PARASITES OF THE FRIT-FLY, *OSCINELLA FRIT* (L.), IN EASTERN NORTH AMERICA.

By F. J. SIMMONDS.

Commonwealth Institute of Biological Control.

In a previous paper (Simmonds, 1952) the biology of some parasites of the frit-fly, *Oscinella frit* (L.), has been described. The investigation was undertaken to obtain biological-control agents that might be useful if introduced into England against this pest, and it was essential also to know something of the inter-relationships of the different parasite species, as such relationships may play a large part in determining which species of a given parasite complex are the most important in controlling the host.

There are two phases of this inter-relationship. In the first place there is the possibility of the avoidance by one parasite of a host individual already parasitised by another species. This avoidance of multiparasitism is obviously of importance, since parasitism of an already parasitised host usually results in the loss of one or both of the parasites in that host and a consequent reduction in the efficiency of the parasite complex as a whole. If multiparasitism does occur, then it is necessary to determine which, if either, of the parasite species survives in order to estimate the relative value of the species involved as controlling agents.

This particular aspect of biological control has been discussed very fully in the past by Howard (1897), Fiske (1910), Pemberton and Willard (1918), Thompson (1923 and 1939), Smith (1929), Lloyd (1938, 1940 and 1942) and others. It is unnecessary to detail the various arguments and examples cited by these authors indicating that multiparasitism results in a lowering of the general degree of parasitism, or, on the other hand, the arguments of others that the loss caused by multiparasitism is more than offset by the greater adaptability to diverse environmental conditions acquired by a parasite complex as a result of the presence of additional primary parasites. Recent work has indicated that avoidance of multiparasitism and superparasitism by ovipositing females is more frequent and widespread than was formerly supposed (see Salt (1932, etc.), Ullyett (1936), Lloyd (1938, etc.), Simmonds (1943)).

In certain instances, members of a parasite complex may never come into contact with one another. For example, an egg-parasite of the *Trichogramma* type can never come into contact with a larval or pupal parasite, so that the question of inter-relationships does not arise. However, in the case of the parasites of the frit-fly, all the species can, at some point, come into contact, and their inter-relationships are important. It has not been possible to work out fully the results of all the possible associations between the various species, but several have been investigated.

The effects of the inter-relationships between individuals of the same species in cases of superparasitism are a special aspect of the more general problem. An investigation of the avoidance of superparasitism and the effects of the latter when it does occur has been conducted with *Spalangia drosophilae* Ashm. and will be described in a separate paper; it suffices here to state that the capacity to avoid superparasitism is very well developed in that species. This phenomenon has not been investigated in detail for the other species of parasites, but as far as can be judged from the larval parasites, and certainly in the cases of *Callitula bicolor* Spin. and *Loxotropa* sp. ? *tritoma* (Thoms.), superparasitism generally results in the elimination of all but one individual, which survives and becomes adult. In the case of the pupal parasites, very heavy superparasitism results in the death of all the parasites within the host.

In discussing multiparasitism, it seems best to follow the host through the stages in which it is liable to attack by the various species of parasites, and to consider the possibilities of attack on a single host individual by two or more species and the results to be expected from it.

In the area near Belleville, Ontario, Canada, frit larvae are liable to attack by *Hexacola* sp. first. In the early summer, the attack by this species is light, and the chance of the same host individual being attacked more than once by the same or another species of parasite is remote. However, during July and August the parasitism by *Hexacola* rises to 30–40 per cent. of the host larvae, so that the possibilities of superparasitism become higher. The results in such cases have not been ascertained. It seems probable that the host larvae are later liable to attack by *Polyscelis* sp.n., and a consideration of field data indicates that this species is not so successful as *Hexacola* and probably succumbs when in competition with it.

On formation of the host puparia there is, in July and August, a situation where between 30 and 40 per cent. are already parasitised by *Hexacola* and *Polyscelis* when they are formed. The larva of the latter emerges from the host soon after formation of the puparium and then completes its feeding within the puparium; it is therefore not in competition with the pupal parasites. From breeding experiments, it is evident that the pupal ectoparasites, *Callitula* and *Spalangia*, are intrinsically superior to the endoparasites (*Hexacola* and *Loxotropa*), and while *Callitula*, owing to its small numbers, probably does not greatly affect the numbers of *Hexacola*, it is possible, on the other hand, that *Spalangia* reduces the efficiency of the larval parasite when total parasitism is high. However, ovipositing *Spalangia* females possess well developed faculties for selecting healthy puparia for oviposition, and multiparasitism is usually avoided in the field. In the laboratory, when only very few hosts were made available to it, *Spalangia* successfully parasitised puparia containing *Hexacola*.

In general, therefore, the larval parasites are inferior to the pupal parasites, *Spalangia* and *Callitula*. However, once the larval parasites have emerged from the host to feed on its remains within the puparium, it is certain that they are largely removed from the chances of multiparasitism owing to the selection of apparently healthy hosts by the ovipositing pupal parasites. *Hexacola* larvae remain within the host longer than do those of *Polyscelis* and the former is therefore more liable to multiparasitism than the latter. In July and August, when parasitism in general is high, it is possible that a slight reduction in the numbers of *Hexacola* is caused by further parasitism.

The inter-relationships of the three pupal parasites have been worked out in greater detail, and are discussed separately below.

1. *Spalangia-Callitula*.

It has been found that *Spalangia* is able to discriminate between a healthy host and one that has been parasitised or paralysed, and that the ovipositing females possess considerable powers of restraint which enable them to avoid parasitising hosts sensed to be unsuitable. However, when suitable hosts are scarce, their restraint breaks down. Thus, while there is considerable avoidance by *Spalangia* of hosts already parasitised by *Callitula*, multiparasitism nevertheless occurs on occasion. Though no extensive experiments have been done with *Callitula*, it seems very probable that it is similar to *Spalangia* in its oviposition behaviour.

The effects of competition between the two species in the same host individual have been investigated along two lines:—

1. Oviposition by both species at the same time.
 - (a) More *Spalangia* than *Callitula* ovipositing.
 - (b) Equal numbers of the two species ovipositing.

- (c) More *Callitula* than *Spalangia* ovipositing.
- (d) Varying numbers of hosts with (a), (b), (c).

2. One species parasitising hosts containing immature stages of the other parasite.

In this way it was hoped to determine the variations, if any, in the inter-relationships between the two species in a number of different situations. Experiments were set up using oviposition vials measuring $4\frac{1}{2}$ ins. \times 1 in., with damp cotton wool and a raisin, as in the breeding of the pupal parasites (see Simmonds, 1952). Puparia of *Drosophila melanogaster* Mg. 24 hours old were used as hosts, and the temperature was kept at 83°F. as it was found that *Callitula* laid more consistently at this temperature.

Five females of *Spalangia* and five of *Callitula*, five of *Spalangia* and one of *Callitula*, and one of *Spalangia* and five of *Callitula* were placed, thus grouped, in three experimental vials, and each group was offered 5, 10, 20 and 40 puparia on four consecutive days. After this, the *Spalangia* and *Callitula* females in each vial were separated and placed in six vials, and 40 puparia were inserted in each vial on each of the next two days to ascertain the number of eggs that could be laid by the females of each species in each vial. These experiments were repeated twice, except those in which 40 hosts were used which were repeated only once.

The results are given in Table I. This Table shows that there is no marked intrinsic superiority of either species. From vials B and C it is seen that the species that preponderates with regard to the number of ovipositing females is the more successful. From vial A, where there are equal numbers of each, it appears that *Callitula* is more successful when hosts are very few, but that when more hosts are available the two species are about equally successful. Columns 5 and 6 show that when ovipositing alone, under the conditions of the experiments, the females of the two species laid on the average about the same number of eggs daily. The oviposition rate was presumably similar, modified by the factor of restraint, when the two species were together. Thus, neither species is intrinsically superior when eggs are laid at the same time on the same individual hosts, except possibly under conditions of high superparasitism, when *Callitula* may be slightly superior.

Two experiments were set up to determine the course of events when one species parasitised a host individual that had been subject to attack by the other. In the first, two vials were prepared as before, one containing a number of ovipositing females of *Spalangia* and the other a number of *Callitula*. Into each were placed ten *Drosophila* puparia, which were left with the parasites for 24 hours, and at the end of this period, the two batches of puparia, now mostly parasitised, were interchanged, and each was exposed for 24 hours to the other species of parasite. This was repeated seven times. The results are shown in Table II.

TABLE II.

<i>Spalangia</i> after <i>Callitula</i>			<i>Callitula</i> after <i>Spalangia</i>		
S	C	D	S	C	D
17	38	15	25	24	21
S= <i>Spalangia</i>			C= <i>Callitula</i>		
			D=dead pupae		

Thus, when *Callitula* parasitises puparia already attacked by *Spalangia*, about equal numbers of the two parasites emerge, but when *Callitula* is the species that attacks first, it is superior to *Spalangia*. It may be that the ability to avoid host individuals that are already parasitised is greater in *Spalangia* than *Callitula*.

As an extension of this, a second experiment was conducted in which each of 12 pairs of *Callitula* females was provided with ten *Drosophila* puparia parasitised by *Spalangia*, and the various batches of puparia differed in the age of the *Spalangia* larvae or pupae within them. There were 12 such age groups as indicated in Table III, in which the ages of the *Spalangia* are given in days after oviposition. Thus, 24 female *Callitula* were used. In order to counteract to some extent the variation in oviposition rates of the pairs of females, the series was duplicated, but those *Callitula* which had been presented with the youngest *Spalangia* were now given the oldest, and the whole series was reversed. The experiment was then repeated, giving four sets of figures. The results are shown in Table III.

TABLE III.

Age of <i>Spalangia</i> stages in puparia—in days					1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13
<i>Spalangia</i>	19	17	8	20	14	16	17	10	11	2	6	3
<i>Callitula</i>	3	10	20	15	21	11	13	19	15	18	12	13
Dead	18	13	12	5	5	13	10	11	14	20	22	24

This experiment shows that *Callitula*, when provided with puparia containing all ages of *Spalangia* larvae and pupae, is able successfully to parasitise a number of them; no stage of *Spalangia* is definitely superior to *Callitula*. When *Callitula* is allowed to oviposit on full-grown *Spalangia* larvae within *Drosophila* puparia the majority of the hosts yield no adult parasites, and most of those that do so produce *Callitula*. In such cases, *Callitula* is directly attacking *Spalangia* larvae and is acting as a secondary parasite.

The inter-relationships of these two parasites may now be summarised as follows. Marked avoidance of multiparasitism is shown by the ovipositing females, particularly those of *Spalangia*, but when scarcity of hosts causes a breakdown of the normal restraint so that both species are present within the same host puparium, neither shows marked superiority over the other. Each is able successfully to parasitise full-grown larvae of the other.

2. *Spalangia-Loxotropa*.

Preliminary experiments were started with these two species, in the same way as for *Spalangia* and *Callitula*. It soon became obvious that even in a variety of circumstances, *Spalangia* was always superior to *Loxotropa* when the two were in competition, and also that there was marked avoidance of multiparasitism by both species.

3. *Callitula-Loxotropa*.

As in the *Spalangia-Loxotropa* experiments, the ectoparasite, in this case *Callitula*, was superior to the endoparasite when the two were in competition, and avoidance of multiparasitism occurred.

Thus, considering the parasite complex as a whole, the ability to avoid super- and multi-parasitism shown by at least some of the species suggests that competition between the various species arises only when parasitism is high. *Spalangia*, by the time (relative to the stage of development of the host) and nature of its attack, is the most likely to produce multiparasitism, but as its capacity for selecting only healthy hosts for oviposition is very highly developed, the effects of multiparasitism are very small even in this case.

Summary.

Studies are reported on the extent and importance of competition amongst larval and pupal parasites of *Oscinella frit*.

In Ontario, 30–40 per cent. of frit larvae are parasitised by *Hexacola* in July–August and superparasitism is thus likely. Subsequent attack by *Polyscelis* may occur, but probably unsuccessfully.

Competition between the larval parasites and the pupal ecto-parasites *Callitula* and *Spalangia* is generally slight, because the former species is not abundant and the latter tends to select only healthy hosts.

Experiments are described to test the inter-relations of the three pupal parasites by exposing puparia of *Drosophila melanogaster* to two species simultaneously and in sequence.

When *Callitula* and *Spalangia* are allowed to oviposit simultaneously, the former is more successful when hosts are relatively few, but otherwise the two species are equally successful. When *Callitula* parasitises puparia recently exposed to *Spalangia*, equal numbers of the two parasites emerge, but in the reverse experiment *Callitula* is superior. *Callitula* is able to parasitise successfully a proportion of puparia already containing *Spalangia* whatever the age of the latter; mature *Spalangia* thus attacked are mostly killed.

Similar comparisons of *Callitula* and *Spalangia* with the endoparasitic *Loxotropa* show that *Loxotropa* is inferior to both the others, but that all three show marked avoidance of multiple parasitism.

In general, there is little interference between the various species in the parasite complex associated with the frit-fly.

The parasite complex associated with the frit-fly appears to be one that is very well balanced, there being little interference between the various species involved. Moreover, when, for any reason, one species does not attain its accustomed degree of parasitism, this is offset by an increase in the numbers of one or more of the other species.

References.

- FISKE, W. F. (1910). Superparasitism: an important factor in the natural control of insects.—J. econ. Ent., **3**, pp. 88–97.
- HOWARD, L. O. (1897). A study in insect parasitism: a consideration of the parasites of the White-marked Tussock Moth, with an account of their habits and interrelations, and with descriptions of new species.—Tech. Bull. U.S. Dep. Agric., no. 5, 57 pp.
- LLOYD, D. C. (1938). A study of some factors governing the choice of hosts and distribution of progeny by the Chalcid *Ooencyrtus kuvanae* Howard.—Phil. Trans., (B) **229**, pp. 275–322.
- LLOYD, D. C. (1940). Host selection by Hymenopterous parasites of the moth *Plutella maculipennis* Curtis.—Proc. roy. Soc., (B) **128**, pp. 451–484.
- LLOYD, D. C. (1942). Further experiments on host selection by Hymenopterous parasites of the moth, *Plutella maculipennis* Curtis.—Rev. Canad. Biol., **1**, pp. 633–645.
- PEMBERTON, C. E. & WILLARD, H. F. (1918). Interrelations of fruit-fly parasites in Hawaii.—J. agric. Res., **12**, pp. 285–295.
- SALT, G. (1932). Superparasitism by *Collyria calcitrator*, Grav.—Bull. ent. Res., **23**, pp. 211–216.

- SALT, G. (1934). Experimental studies in insect parasitism. I-II.—Proc. roy. Soc., (B) **114**, pp. 450-476.
- SALT, G. (1935). Experimental studies in insect parasitism. III.—Proc. roy. Soc., (B) **117**, pp. 413-435.
- SALT, G. (1936). Experimental studies in insect parasitism. IV.—J. exp. Biol., **13**, pp. 363-375.
- SALT, G. (1937). Experimental studies in insect parasitism. V.—Proc. roy. Soc., (B) **122**, pp. 57-75.
- SIMMONDS, F. J. (1943). The occurrence of superparasitism in *Nemeritis canescens* Grav.—Rev. Canad. Biol., **2**, pp. 15-58.
- SIMMONDS, F. J. (1952). Parasites of the frit-fly, *Oscinella frit* (L.), in eastern North America.—Bull. ent. Res., **43**, pp. 503-542.
- SMITH, H. S. (1929). Multiple parasitism: its relation to the biological control of insect pests.—Bull. ent. Res., **20**, pp. 141-149.
- THOMPSON, W. R. (1923). A criticism of the "sequence" theory of parasitic control.—Ann. ent. Soc. Amer., **16**, pp. 115-128.
- THOMPSON, W. R. (1939). Biological control and the theories of the interactions of populations.—Parasitology, **31**, pp. 299-388.
- ULLYETT, G. C. (1936). Host selection by *Microplectron fuscipennis*, Zett. (Chalcididae, Hymenoptera).—Proc. roy. Soc., (B) **120**, pp. 253-291.
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SELECTION OF A BHC-RESISTANT STRAIN OF *DROSOPHILA MELANOGASTER* MG.

By F. J. OPPENOORTH and D. DRESDEN.

Centre for Research on Biocides of the National Council for Agricultural Research, T.N.O., Utrecht.

In a great variety of insects repeated chemical control sooner or later results in decreased susceptibility. Up to now the study of the genetic and physiological differences between susceptible and resistant animals has not been correlated. In those cases in which it has been possible to collect detailed physiological data, as for example on the resistance of *Musca domestica* L. to DDT, practically nothing is known about the genetic aspect, as the insect does not lend itself to a genetic analysis. Consequently, as its genetic structure is so well known, *Drosophila melanogaster* Mg. suggested itself for further experiments on resistance but, since this insect is not the object of control, no resistant strains are found in the field. For this reason strains resistant to γ BHC have been selected in the laboratory and genetic analysis has been started on them. It is intended sooner or later also to make physiological experiments on one of these strains and this particular poison was chosen because other investigations, on its physiological action, are in progress in the laboratory at Utrecht.

Method.

The methods used in this investigation were the same as those described in a previous paper (Dresden & Oppenoorth, 1953). The main points of the contact method of application of the insecticide only are recapitulated here. Flies were introduced into test-tubes, the walls of which were covered with filter-paper on which γ BHC had been deposited by evaporation of an acetone solution.

In the selection experiments the treatment closely followed this method. The flies were brought into contact with the γ -BHC-treated filter-paper, the survivors being used for breeding the next generation. To shorten and simplify the work a few modifications were made: firstly, the insects were aged from 0 to 8 instead of from 2 to 6 days; secondly, they were only taken from two culture bottles instead of from many.

The quantity of poison chosen was such as to produce a kill of 60-80 per cent. Because of the technical modifications mentioned above, even this could not always be attained, conditions in the bottles fluctuating fairly widely and thus considerably influencing the susceptibility. Owing to the small number of bottles used the variations in susceptibility were, of course, conspicuous.

The number of insects used differed considerably between one selection and another, but averaged about 400.

The process of selection does not depend solely on the mortality found in the corresponding selection owing to certain factors. In the first place part of the females were fertilised by males which, since they did not all survive the selection, may have introduced a degree of susceptibility into the next generation. This results in a slower process of selection. In the second place, the susceptibility of females differs from that of the males, so that their respective rates of selection are different. It is not possible to tell how this influences the process of selection in the population as a whole, because the differing susceptibilities of the sexes have not been determined separately.

In order to find out whether differences in homogeneity of the original material have any influence upon the final result of selection, three strains were subjected separately to selection :

1. "Berlin Inzucht" (BI), a strongly inbred laboratory strain ;
2. "Wild₁" (W1), the offspring of about 50 wild flies.
3. "Wild₂" (W2), the offspring of about 700 wild flies.

Results.

The regression lines of W1 and of the 13th and 24th selection from them are shown in fig. 1.

It appears that the first 13 selections produced a resistant strain and that further selection did not increase resistance appreciably.

The two other original strains had susceptibilities different from that of W1. Nevertheless after about the same number of generations resistant strains were obtained which were very similar to the selected W1. There is no evidence therefore of any influence on final degree of resistance of the degree of homogeneity of the original populations. Further experiments have therefore been made exclusively with the selected W1.

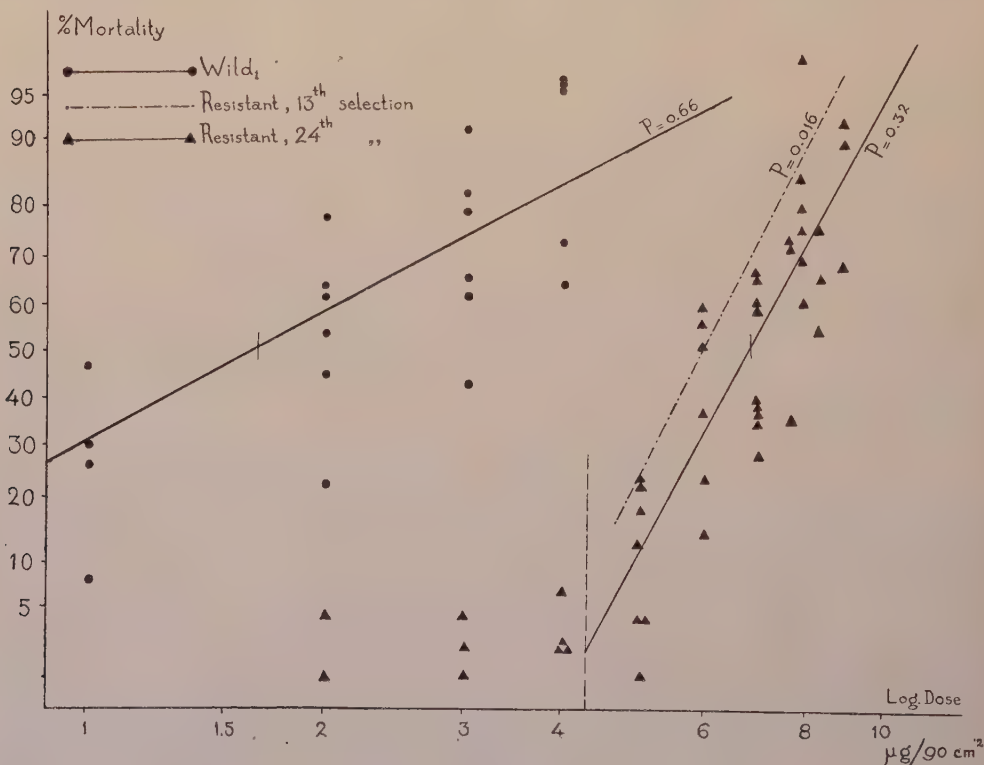


Fig. 1.

Contact method.—P-values calculated from the linearity test. The value "a" was calculated at the dose 1 μg . The constants of the regression lines are expressed as angular-values.

Wild₁ : $a=34$ $s_a=5$ $b=53$ $s_b=12$ $\text{LD}_{50}=1.6 \mu\text{g}$. 95%-Conf. Int. = $1.0-2.0 \mu\text{g}$.

Resistant : $a=108$ $s_a=16$ $b=183$ $s_b=20$ $\text{LD}_{50}=6.9 \mu\text{g}$. 95%-Conf. Int. = $6.6-7.2 \mu\text{g}$.

Ratio at LD_{50} -level : 4.3. The data at the left side of the dotted vertical line are left out of consideration in the case of the 24th (and 13th) selection.

The susceptibility of W1 and its 24th selection to skin-application of the insecticide was determined (fig. 2). Here too, the two strains appear to be different but it is remarkable that in this case the regression lines run parallel, whereas in the contact method they show different slopes. When the insecticide was injected into the body cavity in both strains, a considerable spread of the data appeared, which made the estimation of the regression lines difficult. Nevertheless a significant difference was found between the two strains, the one-sided sign test resulting in a P-value of 0.011 (Table I). It might be thought that the difference in susceptibility between the two strains depended on a difference in their body weights. Therefore samples of the insects were weighed and from Table I it can be seen that no correlation seems to exist. In order to ascertain whether the resistance obtained is specific or whether there is also a decreased susceptibility to other insecticides, a number of experiments was performed to test the susceptibility to DDT and "Thanite" (for Thanite not the kill but the knock-down was determined by counting the number of insects which after a 24 hours' exposure did not fly up within 20 seconds). The resistance appeared to be non-specific. The question of specificity being of only secondary importance to the investigation in hand, these results are omitted. When starting a genetic analysis it should in the first place be determined whether the factors of resistance are exclusively located in the chromosomes and whether they are dominant or not.

TABLE I.
Insecticide injected into the body cavity.

Dose injected in $\mu\text{g}/\text{fly}$	Per cent. mortality		Sign of diff. per cent. mortality W1-Resistant	Av. weight of 20 flies in mg.		Difference in weight W1-Resistant	*
	W1	Resistant		W1	Resistant		
0.0020	5	5		1.10	1.24	-0.14	
	100	0	+	0.65	1.23	-0.58	+
0.0030	60	0	+	1.11	0.95	+0.16	-
	70	5	+	1.20	0.94	+0.26	-
0.0035	40	20	+	0.96	1.15	-0.19	+
	25	35	-	0.84	1.02	-0.18	-
0.0038	55	5	+	1.16	1.25	-0.09	+
0.0040	95	85	+	1.25	0.92	+0.33	-
	100	95	+	1.18	1.13	+0.05	-
	100	90	+	1.15	0.92	+0.23	-
0.0050	100	90	+	0.92	0.86	+0.06	-

*Cases in which a difference in weight may or may not be the cause of difference in mortality are indicated as + and - respectively.

These problems can be investigated by comparing the susceptibility of the ♀♀ of the offspring of reciprocal crosses mutually as well as with the susceptibilities of the parent strains. However, since the latter had always been determined without separating ♂♂ and ♀♀, in examining the susceptibility of the reciprocal F_1 's ♂♂ and ♀♀ were also taken together. This means that an error was introduced into these experiments, because the chromosomal constitution of the ♂♂ progeny is not exactly the same in the two crosses (varying as to the origin of the X and Y chromosomes). For, if the X chromosome contains factors for resistance the ♂♂ from a cross ♀ Resistant \times ♂ W1 will be more resistant than the ♂♂ from ♀ W1 \times ♂ Resistant, irrespective of the question whether or not the resistance factors are dominant. This difference does not exist in the ♀♀.

Meanwhile, the regression lines (fig. 3) show that the offspring of the reciprocal crosses are mutually only slightly different (at least at the LD50 level) and that their susceptibility is closer to that of the resistant parent strain than to that of the

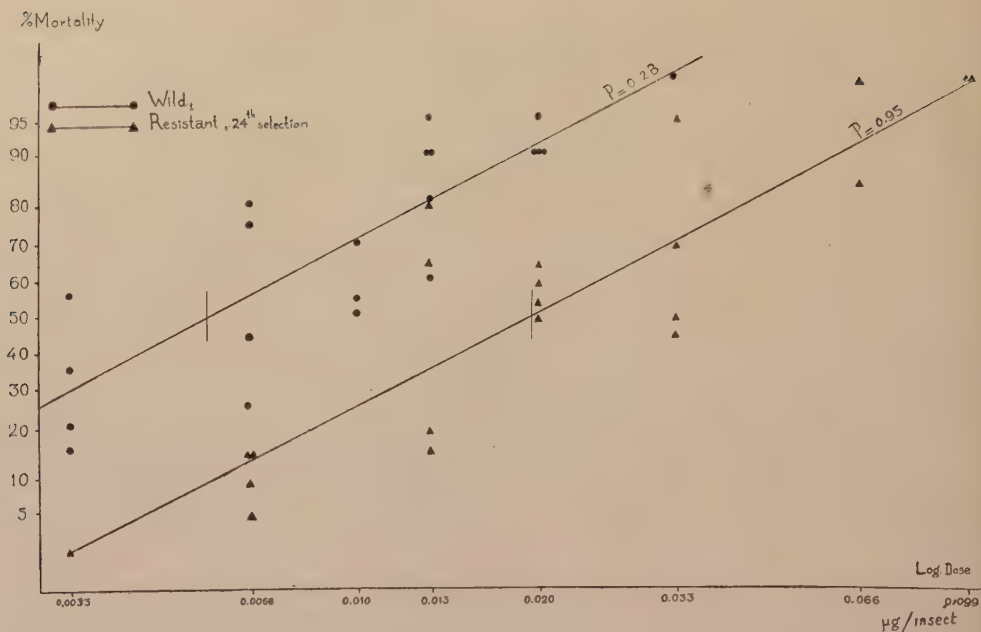


Fig. 2.

Skin application.—P-values calculated from the linearity test. The value "a" was calculated at the dose 0.066 µg. The constants are:

Wild₁: $a=100$ $s_a=6.6$ $b=52$ $s_b=7.5$ $LD_{50}=0.0057$ µg./insect.

Resistant: $a=75$ $s_a=4.0$ $b=53$ $s_b=6.1$ $LD_{50}=0.0182$ µg./insect.

Ratio at LD₅₀-level: 3.2.

susceptible one. It follows that the theoretical objection which may be raised against the method employed has no practical weight in this particular case. Consequently one may conclude that the cytoplasmatic inheritance, if any, is slight, and that the resistance is incompletely dominant.

Discussion.

It is evident that both for genetic and physiological research a wide difference between susceptible and resistant strains is desirable. In this respect the above results are not very promising.

Riemschneider and Rohrmann (1950) selected a strain of *Drosophila* resistant to DDT, Weiner and Crow (1951) selected strains resistant to DDT. They do not present any regression lines, but it appears from the mortality percentages recorded that in their strains, too, resistance did not attain a high level. Similar results were obtained with *Musca domestica* by Lindquist and Wilson (1948) and Bickle and others (1948).

There are, however, resistant strains of *Musca domestica* known, which have reached a far higher level, by selecting in the laboratory (Bruce & Decker, 1950) or by field control. It is not clear whether this high resistance is due to particular properties of the insect or to the manner in which the selection took place.

A genetic change as a result of selection may be based on two mechanisms, firstly, mutation and secondly, an increase of the frequency of the genes concerned already present in a population. We are of opinion that the fact that in our three populations

%Mortality

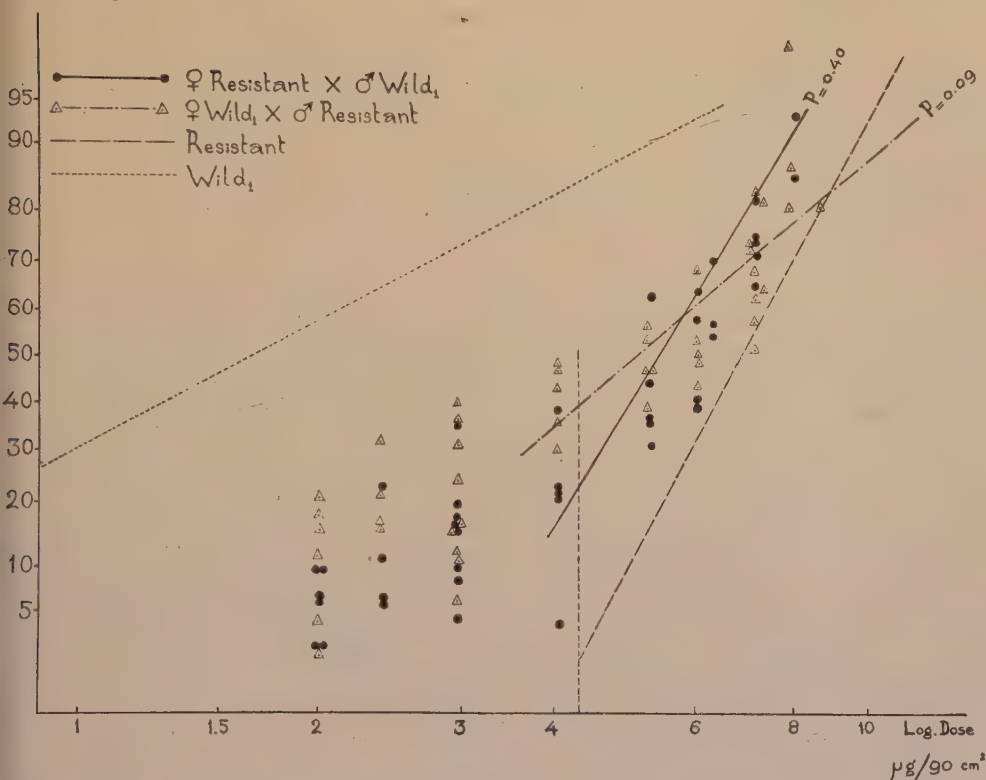


Fig. 3.

Contact method. P-values calculated from the linearity test. The value "a" was calculated at the dose 1 μg . The constants are:

♀ Wild₁ × ♂ Resistant: $a = -12$ $s_a = 12$ $b = 83$ $s_b = 15$ $\text{LD}_{50} = 4.9 \mu\text{g}$. 95%-Conf. Int. = 4.3–5.4 μg .

♀ Resistant × ♂ Wild₁: $a = -82$ $s_a = 13$ $b = 173$ $s_b = 16$ $\text{LD}_{50} = 5.4 \mu\text{g}$. 95%-Conf. Int. = 5.1–5.7 μg .

The data at the left side of the dotted vertical line are left out of consideration.

the same resistance developed in the same way practically excludes the possibility of mutation. The resistance obtained must therefore result from a change of the frequency of one or more of the genes already present. When a resistant strain develops in the field, the chances of mutation playing its part are considerably greater, for then selection takes place from a far larger population over a far longer space of time. As in the present experiments the same rate of resistance was obtained from three different parent strains, it appears that the occurrence of genes responsible for this kind of resistance is by no means an exception. Within the scope of our investigations the course of the development of resistance appears to be very similar to that of the development of polygenic properties by selection in *Drosophila*, e.g., the number of hairs on the abdomen (Mather, 1942) and the number of spines on the scutellum (Sismanidis, 1942). In these examples the effect of selection gradually decreases as the possibilities of recombinations are exhausted or, as far as they still exist, are due to less and less frequent cross-overs. Whether the resistance of the strains in the present work is also polygenically determined can only

be ascertained when more is known about the localisation of such genes. Experiments on this subject are in progress.

It is remarkable that in the contact method the spread of susceptibility in the resistant strain differs from that in the susceptible strain (non-parallel regression lines), whereas in the skin-application method the spreads are the same (parallel regression lines). The selection by the contact method produced differences in susceptibility which also appeared to exist when skin-application was carried out; but in addition, the resistant strain had become more homogeneous as to factors which only assert themselves in the contact method. The cause of this phenomenon is not understood.

Summary.

Two wild strains of *Drosophila* and one laboratory strain were selected for resistance to γ BHC by a contact method. From each of these three strains equally resistant strains developed in about the same time. They did not become more resistant after prolonged selection. The resistance obtained was further investigated in one of the strains.

There was no evidence of any specificity: the susceptibility of the resistant insects to DDT and "Thanite" also appeared to be less than that of the original strains.

Although the strains were selected by a contact method they also showed decreased susceptibility when the poison was applied to the skin and when it was injected. From the reciprocal crosses F_1 's were obtained the susceptibilities of which were practically the same and differed little from that of the resistant parent strain. It follows that resistance does not depend on cytoplasmatic heredity and that it is incompletely dominant.

Acknowledgements.

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References.

- BLICKLE, R. L., CAPELLE, A. & MORSE, W. J. (1948). Insecticide resistant houseflies.—*Soap & sanit. Chem.*, **24**, no. 8, p. 139.
- BRUCE, W. N. & DECKER, G. C. (1950). House fly tolerance for insecticides.—*Soap & sanit. Chem.*, **26**, no. 3, p. 122.
- DRESDEN, D. & OPPENOORTH, F. J. (1953). Residual effect, skin application and injection of γ -hexachlorocyclohexane in *Drosophila melanogaster*.—*Bull. ent. Res.*, **43**, p. 581.
- LINDQUIST, A. W. & WILSON, H. G. (1948). Development of a strain of houseflies resistant to DDT.—*Science*, **107**, p. 276.
- MATHER, K. (1942). The balance of polygenic combinations.—*J. Genet.*, **43**, p. 309.
- RIEMSCHEIDER, R. & ROHRMANN, B. (1950). Über die Zucht DFDT-resistenter *Drosophila melanogaster* M.—*Anz. Schädlingssk.*, **23**, p. 148.
- SISMANIDIS, A. (1942). Selection for an almost invariable character in *Drosophila*.—*J. Genet.*, **44**, p. 204.
- WEINER, R. & CROW, J. F. (1951). The resistance of DDT-resistant *Drosophila* to other insecticides.—*Science*, **113**, p. 403.

TOMATO GALL MITES FROM MOROCCO.

By K. P. LAMB.

*Plant Diseases Division, Department of Scientific and Industrial
Research, Auckland, New Zealand.*

Through the courtesy of M. Perret, le Chef du Service de la Défense des Végétaux, Rabat, Morocco, the author has been able to examine preserved specimens of tomato gall mites from that region. The two species present were: the tomato erineum mite, *Aceria lycopersici* (Wolff.) 1879, and the tomato russet mite, *Vasates lycopersici* (Massee) 1937. This appears to be the first record of the latter species from North Africa.

The synonymy of these mites has already been discussed (Lamb, 1953), but examination of the erineum-mite material has brought to light a further synonym, *Aceria cladophthirus* (Nalepa) 1892. *A. cladophthirus* was described from *Solanum dulcamara* L., on which it caused shoot deformation and erineum formation. The mite was later redescribed and figured by Nalepa (1911) and his description is equally applicable to the Moroccan tomato erineum mite.

In his description of the tomato erineum mite, Massee (1939) described the featherclaw as 3-rayed. This character was used in a previous revision (Lamb, 1953) to separate *A. lycopersici* from *A. cladophthirus*. However, all specimens of the tomato erineum mite recently examined by the author have been found to have the featherclaw 4-rayed, so that the species cannot be separated by this character. In fact, *A. lycopersici* and *A. cladophthirus* are now believed to be identical. The following description has been drawn up from recently examined mites sent from Rabat, Morocco, on 25th June, 1952, and labelled "Erinose velue no. 1".

***Aceria lycopersici* (Wolffenstein) 1879.**

Phytoptus lycopersici Wolffenstein, 1879, Monatschr. Gartenb., **22**, p. 424.

P. cladophthirus Nalepa, 1892, Anz. Akad. Wiss. Wien, **29**, p. 16 (*descr. nulla*).

P. cladophthirus Nalepa, 1892, Denkschr. Akad. Wiss. Wien, **59**, p. 526 (**syn. nov.**).

Eriophyes cladophthirus (Nalepa) 1898, Das Tierreich, Lief. 4, p. 35.

Phytoptus calacladophora ascr. Nalepa (nom. nud.) 1898, Bull. Florida agric. Exp. Sta., no. 47 pp. 143-144.

P. calacladophora Rolfs (nom. nud.) 1907, *ibid.* no. 91 p. 14.

Eriophyes calacladophora Watson (nom. nud.) 1914, *ibid.* no. 125.

Eriophyes lycopersici Massee, 1939, Ann. Mag. nat. Hist., (11) **3**, pp. 617-619.

Aceria cladophthirus (Nalepa) Keifer, 1946, J. econ. Ent., **39**, p. 570.

Aceria lycopersici (Wolffenstein) Lamb, 1953, Bull. ent. Res., **44**, p. 347.

FEMALE (fig. 1). Length 140-200 μ . Body elongate, cylindrical, 54 μ thick. Rostrum 17 μ long. Dorsal rostral setae 6 μ long, terminal rostral setae 1 μ long. Thoracic shield 33 μ wide, 25 μ long, with approximately five longitudinal striae in

mid-field, the lateral fields granular in appearance. Two dorsal-shield setae 45μ long and 29μ apart, located at the rear margin and directed posteriorly.

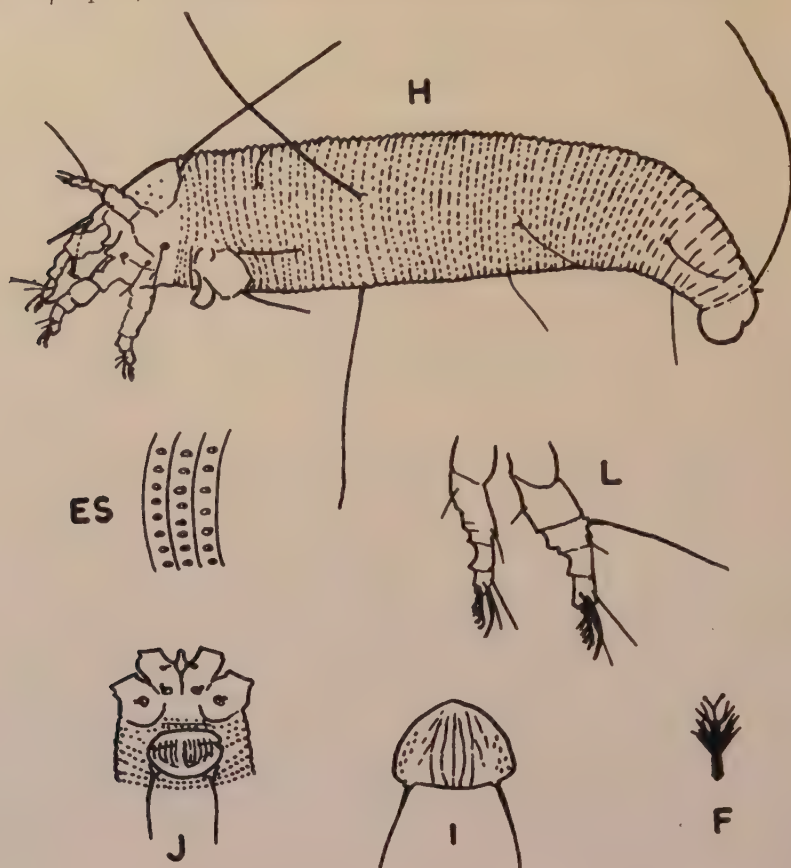


Fig. 1.—*Aceria lycopersici* (Wolff.). (H) adult female, side; (ES) detail of side skin; (L) right legs; (J) female genitalia; (I) cephalothoracic shield of adult; (F) adult feather-claw from below.

Forelegs 28μ long; tibia 7μ long; tarsus 8μ long; claw bristle 8μ long, blunt, moderately curved; featherclaw 6μ long, with 3 compound lateral rays and a bifid tip (*i.e.* 4 rays in all). Hindlegs 23μ long; tibia 6μ long; tarsus 7μ long; featherclaw and claw bristle as above. Ventral thoracic setae I, 7μ long; thoracic setae II, 16μ long; thoracic setae III, 42μ long. Sternum apparently simple.

Abdomen with about 66 uniform, microtuberculate rings. Lateral setae 23μ long, located on 10th abdominal ring. Ventral setae I, 61μ long, located on the 15th post-genital ring; ventral setae II, 16μ long, located on 34th post-genital ring; ventral setae III, 23μ long, located on 5th ring from rear. Caudal setae about 60μ long. Accessory caudal setae stiff, 4μ long.

Epigynum bowl-shaped, 24μ wide, 18μ long. Coverflap longitudinally striated. Genital setae 20μ long.

MALE: Not studied.

Vasates lycopersici (Masse) 1937.

The tomato russet mites received from Rabat were morphologically indistinguishable from local (New Zealand) specimens. The measurements of a

typical Moroccan russet mite are given below. The synonymy of this species has been given in full in a previous paper (Lamb, 1953), so is not repeated here.

FEMALE. Length 194μ . Body phyllocoptiform, 64μ wide. Thoracic shield 42μ long, 55μ wide, with characteristic pattern (fig. 2). Two dorsal-shield setae approximately 12μ long located on the rear margin and directed posteriorly.

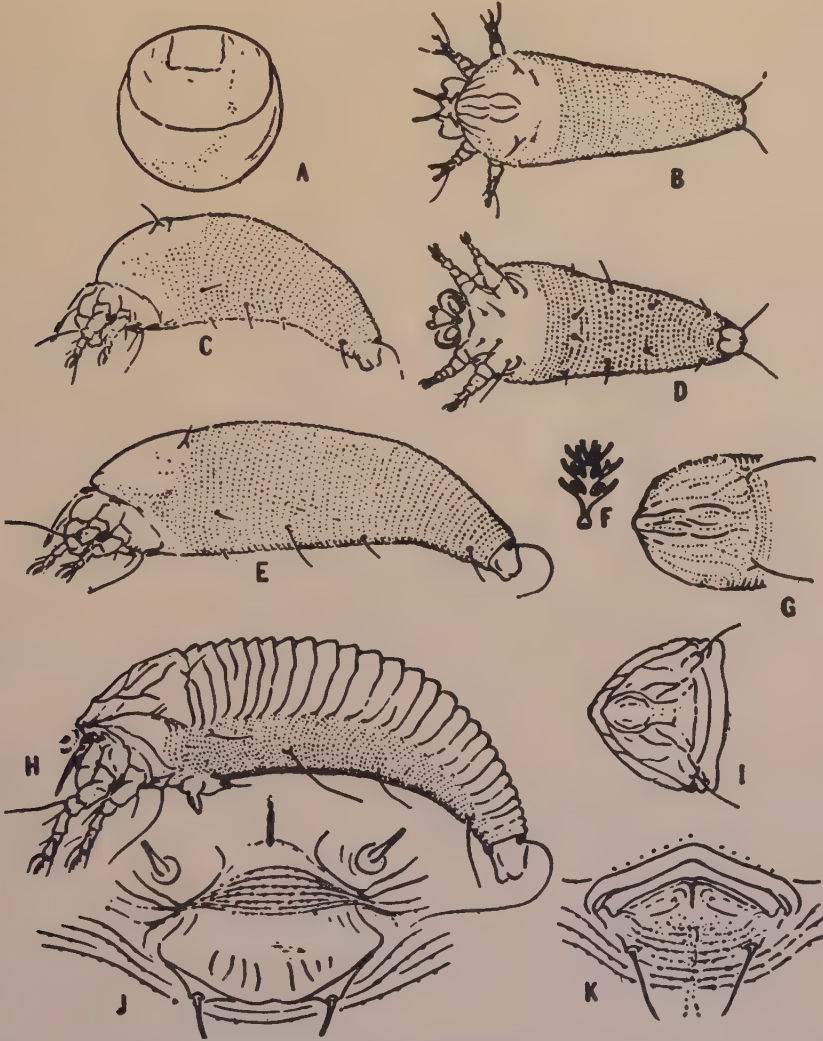


Fig. 2.—*Vasates lycopersici* (Massee) (after Bailey and Keifer). (A) egg; (B) first-stage nymph—dorsal; (C) first-stage nymph—side; (D) first-stage nymph—ventral; (E) second-stage nymph—side; (F) adult featherclaw from below; (G) second-stage nymph—cephalothoracic pattern; (H) adult female—side; (I) cephalothoracic shield of adult; (J) female genitalia; (K) male genitalia.

Forelegs 34μ long; tibia 9μ long; tarsus 8μ long; claw bristle 9μ long, with a terminal knob; featherclaw with 3 compound lateral rays and a bifid tip. Hindlegs 30μ long; tibia and tarsus as above; claw bristle 8μ long; featherclaw

as above. Thoracic setae I, 8μ long; thoracic setae II, 22μ long; thoracic setae III, 44μ long. Sternum simple.

Abdomen with 26 smooth dorsal half rings and 65 microtuberculate, post-genital, ventral half rings. Lateral setae 31μ long, located on ring 8. Ventral setae I, 69μ long, located on 20th post-genital ring; ventral setae II, 22μ long, located on 39th post-genital ring; ventral setae III, 28μ long, located on 6th ring from rear. Caudal setae about 60μ long.

Epigynum bowl-shaped, 24μ wide and 17μ long. Coverflap longitudinally striated. Genital setae 14μ long, inserted posterolaterally.

M. Perret (*in litt.*, 1952) described these mites as "agents d'une maladie de la tomate dénommée ici: Erinose bronzée." They were collected together with specimens of *A. lycopersici*.

It now appears that the two species of mites considered in this paper are almost world-wide in distribution and they probably occur in most countries where tomatoes are grown. The tomato erineum mite has not, however, been recorded from Australia or New Zealand.

This study has been assisted considerably by the use of phase microscopy (Bennett & others, 1951), a technique which lends itself admirably to the study of Acarina in general and gall mites in particular. The best results were obtained with mites mounted in various Berlese fluid formulations, particularly in two media having refractive indices (in the liquid state) of 1.4350 and 1.4698, respectively. In these media, the setae and featherclaws appeared deep black and sharply delineated. Striations on various organs were also more readily resolved than is usually the case with normal microscopical methods.

Summary.

Descriptions are given of the tomato erineum mite, *Aceria lycopersici*, and the tomato russet mite, *Vasates lycopersici*, in Morocco, from which country the latter species is recorded for the first time.

A. cladophthirus is shown to be a synonym of *A. lycopersici*.

These two mites are almost world-wide in distribution and probably occur in most countries where tomatoes are grown, but *A. lycopersici* has not been recorded from Australia or New Zealand.

Acknowledgements.

I wish to thank M. Perret, le Chef du Service de la Défense des Végétaux, Rabat, Morocco, for providing material for study, and also Professor S. F. Bailey, of the University of California, for kind permission to reproduce figure 2 from the *Journal of economic Entomology*.

References.

- BENNETT, A. H., JUPNIK, H., OSTERBERG, H. & RICHARDS, O. W. (1951). Phase microscopy. 320 pp. London, Chapman & Hall; New York, John Wiley & Sons.
- LAMB, K. P. (1953). A revision of the gall-mites (Acarina, Eriophyidae) occurring on tomato (*Lycopersicum esculentum* Mill.) with a key to the Eriophyidae recorded from Solanaceous plants.—Bull. ent. Res., **44**, pp. 343–350.
- MASSEE, A. M. (1939). A species of gall-mite (Eriophyidae) injurious to tomato. —Ann. Mag. nat. Hist., (11) **3**, pp. 617–619.
- NALEPA, A. (1911). Eriophyiden.—Zoologica, **61**, pp. 166–293.

REVIEW OF THE BIOLOGICAL CONTROL OF COCCIDS ON COCONUT PALMS IN THE SEYCHELLES.

By D. VESEY-FITZGERALD.

The introduction of Coccinellid predators for the control of certain Coccids on coconut palms in the Seychelles has already been noted (Vesey-FitzGerald, 1940b). After a lapse of 13 years, the opportunity arose to revisit the islands during 1952 and assess the results obtained from the work. The success has been so complete that a review of the present status of the pests and predators seems to be justified.

Originally the problem had two particularly interesting features. Firstly, several species of Coccids were involved, none of which was recorded as a serious pest of coconuts elsewhere, and secondly, the immediate effect of reducing the incidence of one species was to allow another room to spread on the palms. It may also be recalled that while the main work centred on the control of Diaspid species, certain Lecaniid species and some mealybugs had to be considered because of their part in causing the growth of 'sooty moulds' and encouraging the spread of ants.

Chilocorus distigma (Klug), was introduced from East Africa during 1936 and propagated on *Pseudaulacaspis pentagona* (Targ.) attacking papaya. The beetle became established on *Ischnaspis longirostris* (Sign.) which was at the time the most abundant Coccid on coconut. However, this predator did not seem to be so successful against the smaller *Pinnaspis buxi* (Beh.), which showed signs of replacing *Ischnaspis* on coconut in some places. These indications could not be ignored because, although *Pinnaspis* was not originally so abundant and widespread as *Ischnaspis*, it caused far more serious damage to the palms.

A smaller species of Coccinellid, *Chilocorus wahlbergi* Muls., was also introduced into the Seychelles from East Africa at the same time with the object of seeing if it would attack *Pinnaspis*, but this species never thrived and although it became established for a short time it eventually died out.

Introduction of *Chilocorus nigritus* (F.).

In view of these considerations, a shipment of a third species, *Chilocorus nigritus*, which had been stated to be useful in India, was obtained from Coimbatore at the end of 1938. Forty beetles were received alive in the Seychelles.

C. nigritus was bred on scale-insects attacking the stems of bamboo by the method that had been successfully employed for rearing *C. distigma* on papaya, the latter plant being practically free of them at that time owing to the activity of the previously established predators. After one generation had been bred, liberations totalling some 400 adults were made at several localities in east Mahé, and by the middle of 1939 this species had become firmly established. The beetles immediately started feeding on *Ischnaspis longirostris*, *Chrysomphalus ficus* Ashm. and *Pinnaspis buxi*; the latter was at that time the most abundant Coccid on coconut, *Chilocorus distigma* having by then materially reduced the numbers of the first two.

By 1940, *C. nigritus* had become the commonest introduced Coccinellid, *C. distigma* having waned in abundance as *Ischnaspis* became reduced. During 1944, *Chrysomphalus ficus* reappeared in isolated areas in La Digue, but *C. nigritus* was soon able to control these outbreaks. During 1945 and 1946, outbreaks of scale insects were reported from several areas in the Colony but they

were successfully controlled by *C. nigritus* as soon as the latter had time to multiply.

Since that time to date (1952), only local and short-lived outbreaks have been reported, and these have always been suppressed by predators. In some cases it is claimed that their work has been hastened by the distribution of adults in the infested areas, but this was not really necessary as *C. nigritus* existed everywhere by this time and showed great aptitude for finding its prey. The beetle has been recovered even from small uninhabited islets off the coast of Mahé where it is not likely to have been carried by human agency.

Chilocorus nigritus was found to be firmly established on all the islands visited during the recent survey, namely Mahé, Praslin, La Digue, North Island, Silhouette and Platte Island.

Life-history.

The adult beetle is almost circular in outline, and its average length is 4 mm. It is shining blue-black above and entirely chestnut brown from the ventral aspect; the frons and lateral angles of the prothorax are brown. The sexes are similar in appearance though the brown on the frons and prothorax may be slightly more distinct in the male. There is a marked superficial resemblance between *C. nigritus* and *Exochomus flavipes* (Thnb.), another imported Coccinellid that has become established in the Seychelles, but the latter is more oval in shape, the angles of the prothorax are a redder brown and the thoracic sternites are black.

The eggs are laid singly or in small groups, usually in sheltered places such as under the shields of dead Diaspids. Oviposition continues over a period and the number of eggs laid each day varies. One female laid 13 eggs in five days, but probably a larger output is the rule for young beetles. The egg measures 0.9 × 0.4 mm.; it is dull yellow and minutely punctate. It adheres lightly to the surface on which it is laid or to adjacent eggs.

The newly hatched larva is 1.4 mm. in length and the width of the head capsule is 0.33 mm. Its colour is pale yellow and there are no markings on the body, but the head is slightly darker and the eyes are black. The dorsal surface of the body is armed with six rows of bristles. The four median rows are black and tipped with a black hair which is $\frac{1}{3}$ as long as the supporting bristle, while the two lateral rows are lighter coloured and their terminal hair is white, downwardly directed and proportionally longer.

As the larva grows, a pattern develops. This is quite distinct by the fourth instar. The body in this stage is dull yellow, but the central area of the prothorax, two lateral areas of the meso- and meta-thorax, the fifth abdominal tergite and a small area at the base of each dorsal spine are black. The fifth instar is the last. In this stage, the larva measures 5–6 mm. The head is pale brown and the body buff. The tergites of the meso- and meta-thorax and of the fourth, fifth and sixth abdominal segments have smoky-coloured areas which produce the effect of transverse bands. The body bristles are the same colour as the part of the body from which they arise and are themselves ornamented with long spreading white hairs. The larva is entirely pale buff from the ventral aspect, but the legs are banded and are coloured like the dorsal surface of the body.

The pupa is 4 mm. in length. It is enclosed in the fifth larval skin. It is yellow, but the part which is exposed by the split larval skin is ornamented with black markings, there being two spots on the meso-thorax, a dark band covering most of the meta-thorax, and three dark spots on each of the first six abdominal segments. If several larvae have been feeding together, they pupate together in a group, but the arrangement of the pupae in the group is quite haphazard.

The complete life-cycle occupies about 30 days and the duration of the incubation period, the five larval instars and the pupal period were 5, 4, 2, 2, 4, 5 and 8 days, respectively, in the insectary.

The larvae were found feeding on a great variety of Diaspid, but they were not definitely observed feeding on Lecaniids. However, in cases where the two kinds of Coccids were found together, some of the latter, for example *Eucalymnatus tessellatus* (Sign.) or *Vinsonia stellifera* (Westw.), showed signs of having been attacked. Also, non-breeding adults were often found in considerable numbers on certain plants on which only Lecaniids were present, though proof that the beetles were feeding on these was not established.

Survival of the other imported Coccinellid Predators.

Of the four other predators introduced before the war (Vesey-FitzGerald, 1940b), all except *Chilocorus wahlbergi* were recovered in 1952.

Chilocorus distigma survives as a comparatively scarce insect and was not found on coconut palms. However, it is still widespread and was recovered on *Pseudaulacaspis pentagona* attacking papaya at Grand Anse, Mahé. It was also found on Praslin and on Silhouette associated with the same Coccid.

The two other species that were introduced in 1936 are *Exochomus ventralis* (Gerst.) and *Exochomus flavipes*. They were both recovered in some numbers on Mahé Island, but both species, and especially the latter, are rather easily overlooked owing to their close resemblance to the abundant *C. nigrinus*. The *Exochomus* beetles are predators on Lecaniids and they were found feeding on *Coccus mangiferae* (Green) infesting jak fruit and on another species on *Casuarina*. Many other fruit and timber trees which were severely attacked by soft scales before the war were, during the recent survey, found to be practically free from them, so it may be assumed that these beetles have been instrumental in reducing the infestation. *Eucalymnatus tessellatus* on coconut was also much reduced as compared with pre-war years, but although this Coccid still exists on the palms, the *Exochomus* beetles were never actually found associated with it on this host plant during 1952.

A fifth species, namely *Rodolia cardinalis* (Muls.), was also obtained late in 1938 with the object of trying it against *Icerya seychellarum* (Westw.), which was a minor pest of fruit and timber trees though seldom occurring on coconut palms. In the Seychelles this Coccid was already being preyed upon by *Rodolia chermesina* Muls., but the latter had not established satisfactory control of it, probably on account of interference caused by the ant, *Technomyrmex* (Vesey-FitzGerald, 1940a).

R. cardinalis was introduced from Mauritius. A dozen adults arrived alive and they bred well in the laboratory. During 1939, liberations were made as follows:—on the east coast of Mahé, 100 adults; on Sisters Island, 155 and on Desroches Island in the Amirantes, 65. At Desroches Island the *Icerya* was reported to be prevalent, but *Technomyrmex* did not occur there. It was believed at the time that the species had not become established in the Colony. However, during 1952 the beetle was recovered in great numbers at Grand Anse, Praslin, where it was feeding on *I. seychellarum* attacking young *Casuarina*.

Further search revealed *R. cardinalis* to be widespread in Praslin, Mahé and Silhouette, though no record exists of its being introduced into the latter, which is separated from Mahé, the nearest island where it was liberated, by over 12 miles of sea.

The beetle was always found attacking *Icerya seychellarum* and usually on *Casuarina*, and it seems that the needle-like foliage of this tree enables the Coccinellid to dodge the ant, *Technomyrmex*, to some extent. However, *R. cardinalis* was also recovered feeding on *Icerya* infesting *Citrus*, avocado and jak fruit.

R. cardinalis is now more abundant than *R. chermesina* in the Seychelles and, probably due to the combined attention of these two beetles, *Icerya seychellarum* has apparently diminished somewhat in recent years. However, it is still considered a minor pest of orchards and forest nurseries.

Records of native Coccinellidae.

Nephus oblongosignatus (Muls.).

Common on Mahé Island. Feeds on mealybugs of the genus *Pseudococcus* but does not exert effective control. The larva is ornamented with long, white, waxy filaments and therefore itself resembles a mealybug in appearance.

Sticholotis madagassa Weise.

Common on Mahé and Praslin and apparently native to the Colony. Feeds on stem-infesting Coccids such as the white scale, *Pseudaulacaspis pentagona*, on papaya, and certain species on the stems of bamboo, but does not maintain effective control of them.

Stethorus sp.

This minute black Coccinellid, which is near *S. aethiops* Weise, is apparently native although recorded here for the first time from the islands. It was found quite commonly on the foliage of coconut palms on Praslin.

The beetles frequent the younger leaves and feed on a "red spider". The adults run actively over the foliage and seize the mites with great avidity whenever one is encountered. The mite often springs away from the beetle when the two come into contact and thus saves itself. Also, a web is spun above the surface of the leaf and the mites take sanctuary within it; the Coccinellid never enters this web. Cast skins of the mite were found in the web but never any sign of the beetle. The food of the mites was not ascertained, but they spend much of their time running on the coconut-leaf surface and it is then that *Stethorus* catches them.

No damage to the palm can be attributed to the red spiders and the effectiveness of the control on them by the beetle was not ascertained.

Stethorus pupates on the foliage. The pupa is black and shiny and covered with fine hairs. The larval skin slips right back to the caudal extremity of the pupa and so does not enclose it in any way. The larva was not seen.

Records of Coconut-feeding Coccids in the Seychelles.

During the 1952 survey, the relative scarcity of Coccids on coconut palms as compared with the heavy infestation during the 1930's was most striking. The collection made during the earlier period was unfortunately lost during the war, but it appears that all the species noted formerly can still be found on coconut in the islands though in greatly reduced numbers and never in epidemic form. The few instances of an incipient Coccid epidemic that were examined revealed that more than one species was invariably involved, but the Coccinellid predators were always present and in every case they completely suppressed the outbreak.

The author is very much indebted to Dr. W. J. Hall, C.M.G., M.C., Director of the Commonwealth Institute of Entomology, for determining the Coccid material collected during the recent investigation.

A few species of minor importance are apparently indigenous, but the others are widespread and have certainly been introduced into the Colony. However, it is believed that no additional species has gained a footing since the earlier investigation, and those that have been recorded as dangerous coconut-feeding species in other countries, such as *Aspidiotus destructor* Sign., are still unrepresented in the Seychelles.

Eucalymnatus tessellatus (Sign.) is frequent on the lower surface of the pinnae of older leaves and occasionally forms small colonies. Its occurrence is largely confined to the better palms growing on flat land at sea level. The general incidence of it is, however, much lower than in 1939 and the associated 'sooty mould' is now seldom seen on coconut palms except where they are very closely spaced and overgrown by other trees. The ant, *Technomyrmex detorquens* (Wlk.) (*albipes* (F. Sm.)), is usually found tending this Coccid and the localised distribution of *Eucalymnatus* and the frequent association of Diaspids with it are probably a manifestation of the ant-predator balance. *Chilocorus nigritus* has not been found feeding on *Eucalymnatus* with certainty although adult beetles have been noticed where the latter is present. In captivity, if no other Coccids are offered, the beetles will feed on the younger individuals of this species.

Vinsonia stellifera is fairly frequent, but always thinly distributed, on coconut palms. Examples of this species are often found that appear to have been attacked by a predator, probably *C. nigritus*, though no beetle was ever actually observed feeding on it. *Vinsonia* causes no damage to coconut.

Paralecanium sp. is an Asiatic Coccid which is here recorded from the Seychelles for the first time, but it was certainly present when the scale epidemic was at its height. Solitary on the lower surface of coconut foliage, this species is never abundant or responsible for any damage. Individuals with a parasite emergence hole are often seen but there is no evidence that this species is attacked by the predators.

Pinnaspis buxi can still be found on the foliage of coconut palms but only very occasionally in epidemic form. A few localised outbreaks were examined on Mahé during the recent survey. In one locality, the infested palms were situated in an enclosed stream valley where many other trees and provision crops were also growing. On the younger foliage, the Coccids were not very closely packed, but considerable yellowing of the foliage where they were feeding was characteristic. The older leaves were 'scorched' and the tips of the pinnae rolled. The scales on these leaves were very closely packed but most of them were dead. Another local patch of infestation in Mahé was examined where palms of various ages were growing on alluvium at sea level. On Praslin, *Pinnaspis* was also found on both young and old coconut palms, but only very sparingly. Other Diaspid species, including *Chrysomphalus ficus*, and in one case *Ischnaspis longirostris*, were found associated with *Pinnaspis*, and the ant *Technomyrmex* was abundant sheltering in the rolled pinnae. Wherever *Pinnaspis* was found on coconut, *Chilocorus nigritus* was found in attendance and both adults and larvae were seen to be actively feeding on it. There is no doubt that this predator is keeping *Pinnaspis*, and the other Diaspid scales associated with it, under complete control and that the outbreaks noted were only of a temporary nature.

Ischnaspis longirostris was not found on coconuts at all during the recent survey, except in the single instance associated with *Pinnaspis* as noted above. When it is recalled that this species literally blackened the palms during the late 1930's, its absence must be considered as the most remarkable achievement of the Coccinellid predators. It is still present in the islands on coffee but never in epidemic abundance. This fact is of considerable interest because it has been stated that *Ischnaspis* was originally introduced on this host plant early in the century. *Chilocorus nigritus* was feeding on *Ischnaspis* associated with *Pinnaspis* on coconut, but was not found feeding on them on coffee. This may be explained by the fact that coffee is grown in humid and shady places in the Seychelles where ants are usually particularly abundant.

Chrysomphalus ficus was observed occasionally on coconut, usually in association with other Coccids such as *Eucalymnatus* and *Pinnaspis*. It forms very localised patches of medium density and is in such circumstances responsible for local yellowing of the part of the leaf attacked. *Chilocorus nigritus* feeds on it

and is certainly responsible for keeping it under complete control. No local outbreaks dominated by *C. ficus*, such as were frequent before 1939, were observed during the present survey. On Praslin, small clusters of *C. ficus* were found on foliage of young coconut palms, but *C. nigritus* adults and larvae were always found to be present and feeding on it. On Platte Island, a sand cay 70 miles south of Mahé, *C. ficus* and *Pinnaspis buxi* were found on the foliage of a young coconut palm. *Chilocorus nigritus*, which had been introduced from Mahé by the manager of the plantation, was found actively feeding on the Coccids, both adults and larvae being present.

Chrysomphalus ansei (Green) appears to be an indigenous species. It occurs rather frequently on the foliage of coconut palms, but always in restricted colonies and usually in association with other Coccids. Its attack causes intense yellowing but it is never abundant enough to be responsible for appreciable damage. The Coccinellid predators feed freely on it, but it is worth recording that though this species and *Phenacaspis* were found to be present on coconut before the predators were introduced, neither species was more abundant then than now.

Chrysomphalus dictyospermi (Morg.), which very closely resembles the last species in appearance, has been found only on the spadix and nuts of coconut palms. Although of fairly frequent occurrence, it never causes any damage because it usually occurs only on older tissues. The predators feed freely on it, but in nature the colonies of it are not often attacked, the ant *Technomyrmex* usually being abundant in the crown of the palm.

Phenacaspis inday (Banks), males and females of which occur in small clusters on the lower surface of the pinnae, often close to the mid rib, causes intense local yellowing of the leaf tissue, but is never abundant enough on the palms to cause any damage. *Phenacaspis* is widely distributed in the Colony and has been found on most of the islands that were visited, especially in the vicinity of dwellings. Many of the female scales have parasite-emergence holes, but the Coccinellid predators have not been observed feeding on this species.

Hemiberlesia lataniae (Sign.) has also only been found on the spadix, nut or bracts at the base of the nut, in all cases on ageing tissue. It therefore causes no damage and is in fact never very abundant, though of frequent occurrence and widespread in the granitic islands.

Lepidosaphes duponti Green is an indigenous species which is found on the petioles of coconut palms but never on the foliage. It therefore causes no damage although it is of quite frequent occurrence.

Icerya seychellarum (Westw.) is found very occasionally on coconut palms although early reports of the Department of Agriculture record it as a pest on them. A mealybug, *Pseudococcus* sp., is rather frequently found in clusters on the spadix or under the shelter of the bracts at the base of the nut. Neither of these species is responsible for any damage to the palms.

Incidence of Sooty Mould.

"Sooty mould" was a feature of the vegetation of the Seychelles during the years of the scale epidemic. Its growth was attributed to the splashing of the foliage by "honey dew" excreted by soft scales and mealybugs. The successful reduction of the hard scales (DIASPIDIDAE) by the *Chilocorus* beetles is not therefore supposed to have contributed to the reduction of the mould.

The *Exochomus* and *Rodolia* beetles, which attack soft scales and certain mealybugs, respectively, have not reduced these pests quite so effectively, so although the mould is very much less prevalent than formerly, and coconut palms in particular are now almost entirely free from it, certain fruit trees still support an unsightly growth.

The Coccid species that are mainly responsible for the growth of sooty mould, and the chief trees that suffer are as follows:—

Eucalymnatus tessellatus is common on cinnamon. It occurs on both surfaces of the leaves, especially of trees growing under good conditions of soil and moisture. On eroded hillsides, the cinnamon trees are generally free from it. The ant, *Technomyrmex*, is always abundant and the growth of sooty mould is usually copious, but no apparent damage to the plant results. Predators are never found on the cinnamon bushes, possibly owing to the aromatic nature of the latter, but the Coccids are commonly infected with the fungus, *Cephalosporium*.

Coccus mangiferae Green is a common scale on the foliage of cinnamon, mango, *Eugenia* spp. and jak fruit, and is also responsible for the growth of sooty mould, but apart from this indirect form of damage, the attacked trees do not appear to suffer. It is preyed upon by the *Exochomus* beetles, but seems to be immune from their attention when on aromatic plants such as cinnamon. Also, *Technomyrmex* is always in attendance on it and when the ant is abundant the predators are not found.

Coccus elongatus (Sign.) has been collected on guava, and *Protopulvinaria pyriiformis* (Ckll.) on cinnamon, and there are several other species of soft scales on a variety of fruit and timber trees. In all cases of infestation, a growth of sooty mould develops; ants are invariably numerous and predators consequently absent. *Vinsonia* and *Ceroplastes* are also found on a wide variety of host plants, and although they are seldom very abundant they contribute to the conditions which encourage the growth of the mould. They are frequently parasitised but are only exceptionally attacked by the predators.

Iceya seychellarum has a very wide range of food plants and in some cases causes considerable direct harm. *Citrus* and *Casuarina* are two economic trees which are particularly liable to damage from this insect. Abundance of *Technomyrmex* and a growth of sooty mould are usually associated with such attack and although the *Rodolia* predators are normally present, they never seem to eliminate the *Iceya* colony entirely.

The Effect of scale-insect Control on Copra Production.

The opinion of planters is unanimous that the elimination of Coccids from the coconut palms in the Seychelles has resulted in a marked increase in the crop of the Colony. The difficulty has been to obtain figures which might illustrate the extent of this advantage.

The report on the initial stages of control by the predators (Vesey-FitzGerald, 1940b) noted that although infestation by one species or another had in some localities reached "saturation" point, the palms still produced a crop in the majority of cases. In the case of infestation by *Ischnaspis*, the damage appeared to be limited to a general lowering of the vitality of the palm due to the intensity of the attack. It was presumed that the yield was reduced and there was evidence in some cases that nuts failed to ripen.

Attack by *Pinnaspis* was more serious. It took an acute form and while it lasted the palms in the infested area were blighted, with the result that the foliage withered and the yield was reduced to nil. Occasionally a few palms died, but in general the attack was limited in time and localised in extent. The effect of *Chrysomphalus ficus* was similar, but although it was abundant, its occurrence as the dominant species on the palms was even more local. As has been noted above, the initial reaction following the control of *Ischnaspis* by *C. distigma* was the increase in abundance of these two more harmful species of Coccids, but this adverse tendency never became fully developed owing to the successful establishment of *C. nigrinus*.

Eucalymnatus never caused any apparent direct damage to the palms, but it may be recalled that it was usually robust trees that were most heavily infested by this species. However, the incidence of *Eucalymnatus* was responsible for the growth of sooty mould and it must be assumed that the blanketing effect of the growth reduced the vigour of the palm; certainly the present-day freedom of the palms from this unsightly growth cannot fail to have influenced the opinion of planters in favour of the predators.

The author is very indebted to Mr. D. Bailey, O.B.E., a leading planter in the Colony, for the trouble he has taken to collect and analyse figures relating to the copra production of the islands during the epidemic and scale-free years. These figures appear to give the best picture of the value which may be credited to the predators.

The tonnage of copra exported annually is known from Customs returns and from this the yield of nuts produced can be roughly calculated by making certain assumptions. As a fair average for the islands, it can be taken that one ton of copra represents 7,000 nuts. An allowance must also be made for the nuts consumed locally which during 1927, when the population was 26,770, was estimated to amount to 4,000,000 nuts. Building on this estimate, the number of nuts consumed by the steadily increasing population can be deduced as amounting to 5,400,000 by the 36,128 inhabitants at the present time.

On the above assumptions, the Colony's annual production of nuts can be calculated, in five-year periods for convenience, with the following results:—

1927-1931	39,122,460 nuts
1932-1936	36,563,400 „
1937-1941	38,477,750 „
1942-1946	42,672,490 „
1947-1951	48,911,020 „

If it is recalled that the scale epidemic was at its worst during the period 1932-1937, but practically eliminated by the early 40's, then a comparison of the yield in nuts during the decade (1932-1941), which included the plague years, with the following decade (1942-1951) of scale-free years should give some indication of the benefit obtained from the introduction of predators.

During the former period the colony's production of nuts per annum averaged 37,500,000 and during the latter nearly 46,000,000. The inference which may be deduced from these figures is therefore very satisfactory.

The plantations on the granitic islands were established early in the century and it is believed that no very large areas have come into new bearing during the 20-year period covered by these figures.

The Stockdale Report of 1931 quotes figures from which it can be deduced that the yield from the coral islands amounted to 10,150,000 nuts out of a total for the Colony of 39,122,460. The 1950 records of the Department of Agriculture give the yield of the flat islands as 12,470,000 in a total of about 48,000,000. It is therefore estimated that about one quarter of the Colony's crop comes from the coral islands and in their case new areas have come into bearing during the period under review. However, the scale epidemic did not in general affect these islands, so it may be assumed that the increase in production from the flat islands is due to the yield of these new plantings.

It is an accepted fact that rainfall has a marked effect on the yield of coconut palms, but precise data are lacking. The author is indebted to Mr. W. F. Stephens, C.M.G., who has had long experience as a planter in Mahé, for his views. In his opinion, an annual precipitation of at least 70 inches is necessary for a good crop. During the 1930 decade he enjoyed ample rainfall in eight consecutive years (average for the period 78"), but during the 1940 decade the rains were bad and the minimum requirement was obtained in only one year

(average for the period 57"). These facts suggest that the poor rainfall may have had an adverse effect on the yield of the palms during the scale-free years which have elapsed since the introduction of the predators, and consequently that the increase in crop which has been general for the Colony is in fact due to the reduction of the pests.

The economic value of the control established by the predators is very hard to estimate in terms of cash, but there are certain considerations that may help to give an idea of it. The original cost of the work amounted to only about £3,000. The mean annual increase in crop can be taken, according to the above figures, to amount to about 2,000 tons of copra, and this represents an increase in value of about £100,000 in a single year when the price of copra is taken to be £50 a ton. The price of copra has in fact been very much higher, and the successful control of the Coccids during recent years has therefore enabled the Colony to reap full advantage from peak prices. Moreover, the recent survey has shown that the predators have established themselves in a balanced economy with their prey, such that no damage is caused to the crop, although the Coccids survive in sufficient quantity to support an efficient predator population. There is every reason to suppose that the established predators will act as an insurance against any other coconut-feeding scale that may in the future become accidentally established in the Colony reaching epidemic status. Therefore the value of the control in terms of increased production will almost certainly be permanent.

Summary.

Reference is made to the several species of scale insects which were pests on coconuts and to the Coccinellids which were introduced to combat them.

The introduction and life-history of *Chilocorus nigritus* is described.

The survival of the introduced Coccinellids and the part each has played in controlling the several species of scale is noted.

The habits of some indigenous Coccinellids are recorded.

The coconut-feeding Coccids of the Seychelles are listed and their economic role mentioned.

The incidence of "sooty-mould" and the species of Coccids responsible for its growth are discussed.

An estimate is made of the value of the control effected by the Coccinellids in terms of increased copra production.

Acknowledgements.

Apart from the assistance mentioned in the text, thanks are expressed to Mr. Durocher Yvon, Director of Agriculture, and his staff. Thanks are also due to Mr. E. S. Brown, Entomologist working on the *Melittomma* problem and to his assistant Mr. J. Dookley for the help they gave to an investigation which was not part of their main job. And finally, very warm gratitude is felt towards the planters of the Seychelles for their hospitality and enthusiastic appreciation of the work.

References.

- VESEY-FITZGERALD, D. (1940a). Notes on some Coccinellidae (Col.) from Islands in the Indian Ocean.—Bull. ent. Res., **31**, pp. 191–192.
- VESEY-FITZGERALD, D. (1940b). The control of Coccidae on coconuts in Seychelles.—Bull. ent. Res., **31**, pp. 253–283.
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THE EFFECT OF HYDROGEN CYANIDE ON THE EGGS OF THE COMMON FURNITURE BEETLE, *ANOBIUM PUNCTATUM* (DEG.).

By J. D. BLETCHLY, B.A., B.Sc., F.R.E.S.

*Entomology Section, Forest Products Research Laboratory,
(Department of Scientific and Industrial Research),
Princes Risborough, Bucks.*

Fumigation with hydrogen cyanide is sometimes used for the destruction of wood-boring insects especially in furniture or articles which it may be inadvisable to treat with a liquid insecticide. Its application for the treatment of infested woodwork in buildings is restricted by difficulties of effective sealing and vacation of the premises.

There are few published data on the concentration/time exposures required to kill wood-boring insects in their different stages of development. Nagel (1921) studied the toxicity of this gas to all stages in the life-cycle of the common furniture beetle, *Anobium punctatum* (Deg.), including the eggs but, owing to leakages from the apparatus, his results cannot be considered reliable. Parkin and Busvine (1937) investigated its effects on larvae and adults of *A. punctatum* and *Lyctus* sp. but not on eggs. Gough (1939), working with *Tribolium confusum* (Duv.), found a decreasing order of resistance as follows: pupa, adult, larva and egg, and showed that susceptibility of the egg decreased with age: he pointed out that other workers found not only was this not the case in different species, but the resistance of eggs, compared with other stages, varies according to the species.

In the absence of information on the toxicity of hydrogen cyanide to the eggs of the common furniture beetle, the most frequent cause of woodworm damage in furniture and joinery in the British Isles, this investigation was planned and carried out in co-operation with the Pest Infestation Laboratory (Department of Scientific and Industrial Research).

Technique.

Blocks of Scots pine sapwood (*Pinus sylvestris*), $2 \times \frac{5}{8} \times \frac{5}{8}$ in., were prepared with a muslin egg-laying surface and exposed to beetles: use was made of the information available on this and the length of the incubation period to obtain eggs of different but known ages varying from 8 to 67 per cent. of their development (Bletchly, 1952). For purposes of comparison with the previous investigation by Parkin and Busvine, 99 larvae, obtained from infested wood stored out-of-doors, were arranged in the three larger arbitrary size-groups they employed and were conditioned for 24 hours at 25°C. and 75 per cent. relative humidity in sawdust-filled glass tubes. Both the control egg-laying blocks and larvae were given the same amount of handling as the fumigated specimens. In their work Parkin and Busvine varied both the concentration and the time, but by keeping a constant concentration and varying the time experiments can be simplified. In the present investigation, fumigation was carried out in a steel chamber of 3,000 litre capacity the temperature of which was controlled by a water jacket at 25°C. The concentration of hydrocyanic acid gas was 4.25 mg./litre and was checked by chemical measurements. The chamber was provided with a series of small ports through which the test blocks and the cages containing larvae were introduced

and withdrawn without disturbance of the concentration which had been built up previously. The specimens were removed at intervals, to give 4 concentration/time exposures. A relative humidity of 70 per cent. was maintained.

Concentration 4.25 mg./litre.

Time		Duration of treatment Hours	Exposure (Concentration/time product Mg. hr. per litre)
In	Out		
10.30	20.30	10	42.5
11.26	17.26	6	25.5
12.05	14.35	2½	10.6
15.20	16.20	1	4.3

Larvae, arranged in the three size groups for each treatment, were exposed without sawdust, which might absorb the fumigant; afterwards they were returned to the sawdust-filled tubes and examined for signs of injury or death and the degree to which they were affected. After exposure both larvae and egg-laying blocks were kept at 25°C. and 75 per cent. R.H., examined twice a week for three weeks and then less frequently for a further nine weeks. Three months after laying, all unhatched eggs were dissected and classified as (1) fertile but unhatched, (2) doubtfully fertile, or (3) infertile. As in previous studies of fumigated larvae, many specimens classified as doubtful remained life-like but motionless for periods up to three weeks and none could be termed dead until post-mortem signs appeared.

Results.

The data obtained for eggs are presented in the following Table; eggs of a similar range of development were used for each exposure. The large number of "doubtfully fertile" eggs among those fumigated is due to the inclusion of eggs at an early stage of development which, on dissection, yielded no definite evidence of fertility. It is assumed, however, that the fertility of the fumigated eggs was of the same order as the controls, *i.e.* 95 per cent.

EGGS

Exposure (Concentration/time product Mg. hr. per litre)	Total	Fertile	Doubtfully fertile	Hatched
42.5	63	54	8	0
25.5	37	14	21	0
10.6	59	44	14	0
4.3	72	64	6	0
Control	38	35	1	33

Complete kill was obtained for eggs irrespective of the stage of development at all exposures, but the minimum lethal treatment was not determined.

The following Table summarises the results obtained with the larvae, which are classified as dead, doubtful or alive; some of those recorded as doubtful in the Table recovered, but the others eventually showed post-mortem signs and a final classification of condition was then made.

LARVAE

Exposure (Concentration/time product Mg. hr. per litre)	Number exposed	Condition					Remarks
		Immediately after treatment			Final		
		Dead	Doubt- ful	Alive	Dead	Alive	
42.5	19	5*	14	0	19	0	*1 probably killed by mites.
25.5	20	5	15	0	19	1	1 recovered after 8- 11 days.
10.6	21	9	12	0	19	2	2) Recovered after 1) 5-7 days
4.3	19	9	9	1	17	2	
Control	20	10†	0	10	10	10	†6 probably killed by bites or injured in handling.

Results for the larvae confirm in general those previously obtained by Parkin and Busvine but in the present series there was survival at a higher concentration/time exposure (25.5 against 17.89) and only the highest figure secured a complete kill. These authors concluded that larger *Lyctus* larvae were more resistant than smaller ones and the present investigation indicates this may also be true of *A. punctatum*, since the survivors were in the two larger of the size-groups used. The high mortality amongst control larvae appeared to be due to injuries in three instances and to bites from other larvae in three others. Similar injuries were not found amongst the fumigated larvae. Over a period of 84 days, during which the larvae were kept under observation, the controls lived longer than those which recovered from the fumigation treatments.

It is clear that the eggs of the common furniture beetle are less resistant to hydrogen cyanide than the larvae and it may, therefore, be assumed that commercial dosages which are adequate to kill larvae *in situ* will also kill the eggs which are normally laid on the end grain surfaces, old flight holes, checks and similar crevices.

Summary.

The effect on the eggs of the common furniture beetle, *Anobium punctatum*, to fumigation with hydrogen cyanide at a concentration of 4.25 mg./litre and four concentration/time exposures, varying from 4.3 to 42.5 mg. hr. per litre has been determined. Complete mortality was obtained in all cases. Comparison with data available for larvae show that the eggs are less resistant, from which it is concluded that commercial treatments adequate for infested wood will also kill eggs.

Acknowledgements.

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References.

- BLETCHLY, J. D. (1952). A summary of some recent work on the factors affecting egg-laying and hatching in *Anobium punctatum* De G. (Coleoptera-Anobiidae).—Trans. IXth int. Congr. Ent., Amsterdam 1951, **1**, pp. 728–734.
- GOUGH, H. C. (1939). Factors affecting the resistance of the flour beetle, *Tribolium confusum* Duv., to hydrogen cyanide.—Ann. appl. Biol., **26**, pp. 533–571.
- NAGEL, W. (1921). Bekämpfung von *Anobium striatum* Oliv. mittels Cyanwasserstoffgasen.—Z. angew. Ent., **7**, pp. 340–348.
- PARKIN, E. A. & BUSVINE, J. R. (1937). The toxicity of hydrogen cyanide to certain wood-boring insects.—Ann. appl. Biol., **24**, pp. 131–143.
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TREES AS HABITATS OF THE FOWL TICK, *ARGAS PERSICUS* (OKEN).

By M. ABDUSSALAM and M. M. SARWAR.

Punjab Veterinary College, Lahore, Pakistan.

P. L.

The fowl tick, *Argas persicus* (Oken), is commonly found in chicken houses and in cages in which fowls and other domestic birds are kept; in Iran and Baluchistan it occurs in human dwellings too (Montgomery, 1908). It is also known to occur on trees used as chicken roosts (Schanph, cited by Nuttall & others, 1908), but has not hitherto been recorded from trees that are not so used. Recently, however, it has been found on many trees on which chickens have never perched but which are used as roosting and nesting places by certain wild birds. This finding is being recorded in the expectation that similar infestations will be found in other parts of the world where this pest occurs.

The attention of the authors was drawn to the presence of the tick in crevices in the bark of a pipal tree (*Ficus religiosa*) in the grounds of the Veterinary College, Lahore, in April, 1952. During that month, and the first week of May, 266 trees belonging to 16 species were examined at Lahore and Sheikhpura. Those examined were of varying ages, but none of them was too small to serve as a perch for birds. Of these trees, 49 (18.4 per cent.) were found infested with all stages of *A. persicus* in crevices and under loose portions of the bark. Specimens examined were identical in structure with examples collected from fowl houses and agreed with the description given by Nuttall & others (1908). All the infested trees were used for roosting and nesting by vultures (*Pseudogyps bengalensis*), and common herons (*Ardea cinerea*) or both. Other birds which used the infested trees for perching were kites (*Milvus migrans*), green parakeets (*Psittacula krameri*), house sparrows (*Passer domesticus*), common mynahs (*Acridotheres tristis*), blue pigeons (*Columba livia*), ring doves (*Streptopelia risoria*), crows (*Corvus splendens*), red-vented bulbuls (*Molpastes cafer*) and, rarely, starlings (*Sturnus vulgaris*). Palm squirrels (*Sciurus palmarum*) lived on most of the large trees examined. A kite chick which had fallen from a nest in an infested tree and an adult vulture which had been shot down, carried larvae of *A. persicus* on their bodies.

The forks of the upper branches where vultures and herons perched harboured the largest numbers of ticks, but on trees with relatively smooth bark and few cracks, the ticks extended down the trunk almost to the ground. On trees with cracked bark, such as shisham (*Dalbergia sissoo*), they were restricted to the upper branches near the perching places. Table I shows the various species of trees examined and those that were found infested. It is considered that the infestation of a tree is determined by its suitability as a roosting place for vultures and herons.

Lahore and Sheikhpura, the two towns where the trees were examined, are 24 miles apart. Since the incidence of the tick was similar in both places, the figures for the two towns are combined.

Many of the ticks collected contained fresh avian blood in the gut. Six of a batch collected from a *Ficus* tree on which vultures were perched were crushed, and a suspension of them in saline was injected intramuscularly in a hen. During three weeks of observation, the hen showed no blood parasites.

TABLE I.

Tree	Number	
	examined	infested
<i>Ficus religiosa</i> (Pipal)	70	9
<i>Ficus bengalensis</i> (Banyan)	3	—
<i>Ficus rumphii</i> (Pilkhan)	3	—
<i>Dalbergia sissoo</i> (Shisham)	64	8
<i>Zizyphus jujuba</i> (Ber)	1	—
<i>Terminalia arjuna</i> (Arjun)	11	3
<i>Acacia arabica</i> (Babul)	19	8
<i>Azadirachta indica</i> (Neem)	2	1
<i>Melia azadarach</i> (Persian Lilac)	1	—
<i>Bauhinia variegata</i> (Kachnar)	1	—
<i>Eugenia jambolana</i> (Jaman)	18	5
<i>Cordia myxa</i> (Lasura)	1	—
<i>Psidium guayava</i> (Guava)	53	14
<i>Mangifera indica</i> (Mango)	5	1
<i>Morus alba</i> (Mulberry)	8	—
<i>Albizia lebbek</i> (Siris)	6	—
	266	49

Discussion.

The occurrence of *A. persicus* in various habitats, including the bark of trees, has hitherto been attributed to domesticated birds, particularly fowls. In rare instances, the larvae of this tick have been seen on wild birds such as the secretary bird (*Sagittarius serpentarius*) and the guinea-fowl (*Numida papillosa transvaalensis*) of South Africa (Bedford, 1932) and the mourning dove (*Zenaidura macroura*) of North America (Nuttall & others, 1908), but it has not been realised that infestations can be derived from sources other than poultry. The finding of colonies comprising all stages of the tick on a considerable number of trees which had no association with poultry shows that the pest thrives in nature quite independently of domestic birds. Indeed, it now seems likely that the incidence of the tick on trees frequented by vultures or herons is much higher than in chicken houses in the same area. It appears that there are rich sources of the pest dangerously close to poultry farms and chicken houses.

Summary.

All stages of *Argas persicus* were found in crevices in the bark of 49 of 266 trees belonging to 16 species in West Pakistan. These trees had not been used as roosts by domestic birds. The infestation seemed to be restricted to those trees used by vultures (*Pseudogyps bengalensis*) or herons (*Ardea cinerea*) for nesting or roosting. Several of the ticks collected contained recently sucked avian blood in the gut but intramuscular injection of crushed material from the ticks into a hen produced no blood infection.

Infested trees may be important as sources of the pest near poultry farms and chicken houses.

References.

- BEDFORD, G. A. H. (1932). 18th Rep. vet. Res. S. Afr., pp. 223-523.
 MONTGOMERY, R. E. (1908). J. trop. vet. Sci., **3**, pp. 1-12.
 NUTTALL, G. H. F., WARBURTON, C., COOPER, W. F. & ROBINSON, L. E. (1908). Ticks, a Monograph of the Ixodoidea. Part I. Sect. I. Cambridge Univ. Pr.

DDT-RESISTANCE IN *PLUTELLA MACULIPENNIS* (CURT.) (LEP.) IN JAVA.

By G. W. ANKERSMIT.

E. M. N.

*Institute for Plant Diseases and Pests, Bogor, Indonesia.**

The diamond-back moth, *Plutella maculipennis* (Curt.), is next in importance to *Crocidolomia binotalis* Zell. among the pests of cabbage in Indonesia. Control measures have been applied for a long time. About 30 years ago lead arsenate was in use, but about ten years later it was replaced by derris with great success. After the 1939-45 war, DDT became available and soon proved to be highly effective. Spraying with DDT rapidly became a common practice among the cabbage growers, especially near Bandung in Lembang (1,250 m.). It is not possible to make any exact statement about its first application in this district, but it is certain that by the end of 1948 DDT was used in large quantities in the Lembang area. Treatments in the field began about five days after planting, and were repeated 14 days later and then every ten days until two weeks before harvest, about 1 kg. actual DDT being applied per hectare at each application. A total of 5 or 6 applications or 5-6 kg. DDT per hectare sufficed to keep the cabbages free from attack by insect pests.

At the beginning of 1951, the cabbage growers noticed that these numbers and concentrations of the DDT treatments no longer gave satisfactory control of *P. maculipennis*. They therefore increased the quantity of DDT per application and nearly doubled the number of applications, with the result that about 45 kg. of DDT per hectare were applied during the whole growing period of the cabbage. Soon, however, this quantity also proved ineffective and a 1 per cent. lead arsenate suspension was added at each treatment and later a small amount of Shelltox (DDT in kerosene) also. Despite the application of these enormous quantities of insecticides, the results were rather poor. The use of DDT was generally abandoned in November-December, 1951, and BHC sprays or dusts were applied with good results. However, BHC may affect the taste of the cabbage, and also that of the potatoes which are grown once a year in the cabbage fields, the annual rotation being cabbage—corn—potatoes. It therefore seems inadvisable to use BHC.

Investigations were started in May, 1951, to ascertain whether *Plutella* had indeed become resistant to DDT and to devise suitable control measures against it. Laboratory experiments were carried out in Patjet (at 1,100 m. on Mt. Gedeh) and field plot trials were laid out in Lembang.

The author is indebted to Dr. H. Vos, entomologist, and Mr. D. Sukarna, plant pathological assistant, of the Institute for Plant Diseases and Pests, for their kind assistance during the work.

Methods and Materials.

In order to investigate the differences in susceptibility to DDT, specimens of *Plutella* from Lembang were compared with others from Patjet, where no difficulties with DDT had been observed at that time. The tests were carried out in the laboratory.

* Now at the Instituut voor Plantenziektenkundig Onderzoek, Wageningen, Netherlands.

The apparatus in these experiments was developed by Fransen (1937). Only dusts can be tested with it. It consists of a small wooden box (25 × 25 cm. and 100 cm. high), closed at the top by two copper sieves with a mesh of 200 microns. A charge of 150 mg. of a dust is scattered as evenly as possible on the upper sieve and brushed through. It falls upon the second sieve and is brushed through this also, so that it descends upon the insects, which are placed in a petri dish in a tray at the bottom. After two minutes the insects are removed, placed in clean petri dishes and provided with food.

Mortality counts were made daily for four days. The total kill for the four days is given as a percentage in the Tables. The experiments were carried out with second-instar larvae of equal size and six days old. Every test was repeated five times, and five larvae were used for each test. It is clear that only the contact effect of the materials was evaluated during these experiments. No laboratory tests with sprays were carried out, as it was not possible to bring the necessary equipment for this work to Patjet.

In the field experiments, randomised blocks were laid out. Known quantities of dust or spray were applied evenly with hand dusters or low-pressure knapsack sprayers. Each plot was completely surrounded during the applications by a screen some 2 m. high, in order to prevent the dust or spray from drifting away and thus contaminating adjacent plots.

Commercial dusts, wettable powders and emulsions were used. In the laboratory tests, the dusts were diluted with talc; the various dilutions are given in the Tables. The controls were dusted with talc only.

Laboratory Trials.

Plutella maculipennis was bred for several generations in the laboratory from specimens collected as larvae in Lembang and Patjet. Tests were conducted to ascertain the susceptibility of larvae of various generations to certain insecticides. The concentrations applied and the percentage kills are shown in Tables I and II below.

TABLE I.
Percentage mortality of *Plutella* larvae of the Lembang strain (L) and the Patjet strain (P) to DDT.

Generation	II		III		V		VIII	
Date of treatment ..	11.vi.51		21.vi.51		21.vii.51		29.ix.51	
	L	P	L	P	L	P	L	P
DDT 5% dust	20	100	28	100	23	84	64	96
" " " to talc, 1:1	0	100	12	100	8	58	20	72
" " " " " 1:3	0	88	4	80	14	56	12	48
" " " " " 1:7	0	68	0	76	5	19	4	24
Control (talc)	0	0	0	0	0	0	0	0

These results show that the Lembang strain was significantly less susceptible to DDT than the Patjet strain. It seems, however, that the difference was smaller in the eighth generation.

Further experiments were conducted after a new sample of pupae had been collected in Lembang in September, 1951. The susceptibility of larvae derived from these pupae was compared with that of larvae derived from Patjet material in tests with DDT, derris (8.3 per cent. rotenone), BHC and toxaphene.

TABLE II.

Percentage mortality of *Plutella* larvae of the Lembang strain (L) and the Patjet strain (P).
(Dates of treatment: 13.x.1951 (I); 21.x (II); 5.xi (III); 6.xi (IV); 20.xi (V).)

Generation	I			I		II	
	L	P		L	P	L	P
DDT 5%	24	96	Derris	96	92	92	100
.. .. to talc, 1 : 1 ..	8	88 to talc, 1 : 1 ..	72	48	44	68
.. .. " " 1 : 3 ..	0	88 " " 1 : 3 ..	52	44	36	36
.. .. " " 1 : 7 ..	0	44 " " 1 : 7 ..	28	36	8	16
Talc	0	0	Talc	0	0	0	0

Generation	III			IV	
	L	P		L	P
BHC 5%	82	100	DDT 5%	16	92
.. .. to talc, 1 : 1 ..	64	100 to talc 1 : 1	12	60
.. .. " " 1 : 3 ..	64	96 " " 1 : 3	12	52
.. .. " " 1 : 7 ..	56	92 " " 1 : 7	4	12
Talc	0	0	Talc	0	0

Generation	III		V	
	L	P	L	P
Toxaphene 20% to talc, 1 : 3	72	88	72	92
.. .. " " 1 : 7	60	68	48	60
.. .. " " 1 : 15	44	56	52	46
.. .. " " 1 : 31	24	40	32	36
Talc	0	0	4	0

These figures, too, show clearly the difference in susceptibility to DDT between the larvae of the Lembang strain and those of the Patjet strain. Furthermore, in the tests with BHC and toxaphene the mortality of Lembang larvae seems to be somewhat lower than that of Patjet larvae. In this series of experiments, there was certainly no difference between the two strains in their susceptibility to derris.

Field Trials.

A field test in six replications was laid out in Patjet in 1950. The plots were planted with cabbage in the form of cuttings, in accordance with local practice. Each plot contained 60 plants, and the treatments were with DDT, BHC and toxaphene dusts. One day before application, on the dates given in Table III,

TABLE III.

Field experiment in Patjet. (Larvae non-resistant.) Number of larvae on 60 plants
(ten plants per plot).

Treatment	Date of counts (1950)						
	3.x	19.x	2.xi	16.xi	30.xi	14.xii	28.xii
DDT 5% dust	10	13	32	157	45	18	5
BHC	6	43	73	250	258	87	12
Toxaphene 20% dust, 1 : 3	5	29	70	210	161	18	6
Untreated	8	32	167	287	319	883	157

counts were made of the number of *Plutella* larvae on ten plants in each plot. In all, seven counts and six applications were made. The quantity of dust used per plot in the first, second, and third applications was 40, 60 and 80 gm., respectively, and in each of the remaining three it was 80 gm. as in the third.

These figures show that DDT dusts gave good control of *Plutella* near Patjet in 1950, toxaphene and BHC being less effective. Other experiments confirmed the good results obtained with DDT treatments (Ankersmit & van der Laan, 1951).

In Lembang the situation proved to be quite different. A field test was arranged in 1951, with weekly applications. The plots were planted on 7th December with cabbage of the variety "Roem van Enkhuizen" sown on 14th November. The spacing was 70 x 70 cm. and the number of plants per plot was 80. The plots were arranged in randomised blocks with six replications of each treatment. Counts were made weekly, one day before application, on ten plants in each plot.

The results and the other experimental data are shown in Table IV. The quantity of dust or spray in the first, second and third applications was 40 gm. or 1 litre, 60 gm. or 1½ litres, and 80 gm. or 2 litres, respectively, and in each of the remaining applications it was the same as in the third

TABLE IV.

Field experiment in Lembang. (Larvae resistant.) Number of larvae on 60 plants (ten plants per plot).

Treatment	Date of counts (1951-52)								
	11.xii	18.xii	25.xii	1.i	8.i	15.i	21.i	28.i	5.ii
35% DDT W.P., 0.3% ..	34	61	276	318	338	690	2027	3317	2746
5% DDT dust ..	57	30	281	298	447	749	1556	2425	2279
20% DDT emulsion, 0.4% ..	35	24	163	152	194	456	2068	2748	3228
7% gamma BHC emulsion, 0.15% ..	50	1	188	72	54	247	501	719	1092
60% toxaphene emulsion, 0.13% ..	28	7	6	5	19	92	182	218	245
Derris dust (8.3% rotenone), 1 : 5 with talc ..	39	19	117	87	60	123	229	324	292
Pyrethrum dust (1% pyrethrins), 1 : 2 with talc ..	53	33	214	202	318	491	1529	1861	2217
Untreated	27	72	361	340	509	564	2085	2481	2283

The plots were harvested on 22nd and 29th February. The results are shown in Table V.

TABLE V.

Yield in kilograms per plot in the Lembang experiment.

Treatment	Yield in kg.	Comparison with untreated
35% DDT W.P., 0.3%	30.6	- 3.2
5% DDT dust	37.8	+ 4.0
20% DDT emulsion, 0.4%	32.9	- 0.9
7% gamma BHC emulsion, 0.15% ..	66.8	+ 33.0
60% toxaphene emulsion, 0.13% ..	88.5	+ 54.7
Derris dust (8.3% rotenone), 1 : 5 ..	82.2	+ 48.4
Pyrethrum dust (1% pyrethrins), 1 : 2	34.0	+ 0.2
Untreated	33.8	—

Limit for significant difference 11.0

Limit for highly significant difference 16.7

These figures show that toxaphene emulsion gave the best control, and that derris was nearly as good and BHC effective, but that the various DDT treatments as well as pyrethrum had no effect on *Plutella*.

The effect of the treatments is also shown in Plate VIII.

Conclusion.

Control of the DDT-resistant *Plutella* near Bandung can be obtained by toxaphene or derris. It seems advisable to use toxaphene during most of the growing period, while for the last applications derris should be used. Derris has the advantage of being less dangerous to the consumer; moreover, Table II indicates that the development of resistance to DDT is not associated with resistance to derris. Table II also suggests that resistance to toxaphene is already developed to some extent, and it is to be feared that toxaphene also may become useless. The use of derris may delay this development.

This instance of resistance to insecticides shows once more the danger of relying completely upon chemical control measures. In tropical countries, where there is often a larger number of generations per year than in temperate regions, this problem may sooner become of importance. As DDT was used in large quantities in the Lembang area by the end of 1948 and the difficulties began in the beginning of 1951, the resistance developed in about two years. The life-cycle of *P. maculipennis* near Lembang takes 20–25 days, which means that there are 15–18 generations per year. About 30–40 generations were therefore needed for the development of DDT-resistance. In temperate regions, the number of generations per year varies between two and six, and consequently the time necessary for the development of DDT-resistance will be much longer than in the tropics. In view of these considerations, it seems advisable not to overlook the various cultural control measures; notably crop rotation, or the possibilities of biological control.

Summary.

The diamond-back moth, *Plutella maculipennis*, and *Crocicidolomia binotalis* are the main pests of cabbage in Indonesia. Until 1951, good control was obtained with DDT sprays and dusts, but in that year growers near Lembang (Bandung) obtained poor results with DDT against *P. maculipennis* as compared with previous years. Investigations were started in Lembang, and also in Patjet (on Mt. Gedeh) where no difficulties had arisen. In laboratory experiments, a marked difference in susceptibility to DDT between a Lembang and a Patjet strain of *P. maculipennis* was demonstrated.

In field tests near Patjet with DDT, BHC and toxaphene, DDT gave satisfactory control and was better than the other two. At Lembang, however, where these three materials were again tested, but with derris and pyrethrum also, toxaphene was the most effective, followed by derris and BHC, while DDT, and also pyrethrum, gave almost no control.

For the time being, toxaphene and derris are recommended, the former during the growing season and the latter subsequently, but it is feared that resistance to toxaphene will develop. It may become necessary to resort to cultural and biological measures.

References.

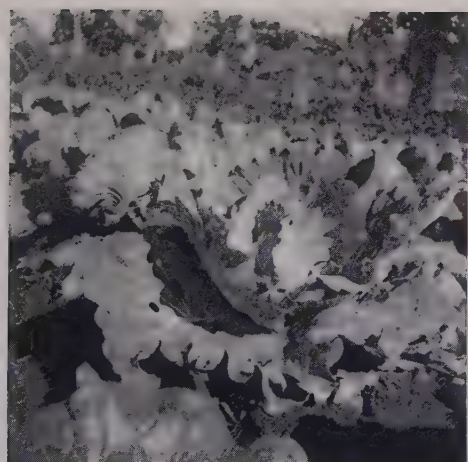
- ANKERSMIT, G. W. & van der LAAN, P.A. (1951). Resultaten van proeven met insecticiden ter bestrijding van insectenplagen in de landbouw in Indonesië.—Landbouw, **23**, pp. 423–484. (With English summary.)
FRANSEN, J. J. (1937). De gevoeligheid der overwinterende rupsen van de bastaardsatijnvlinder voor poedervormige contactvergiften.—Tijdschr. ned. Heidemaatsch., **49**, pp. 203–216.



A



B



C



D

Control of DDT-resistant *Plutella* on cabbage in Java.

- A. Severe damage in a plot treated with a 35 per cent. DDT wettable powder at 0.3 per cent.
- B. Similar damage in an untreated plot.
- C. Satisfactory control by a 60 per cent. toxaphene emulsion at 0.13 per cent.
- D. Satisfactory control by a derris dust (8.3 per cent. rotenone) at 1.5 in talc.

FIELD OBSERVATIONS ON THE CACAO MIRIDS, *SAHLBERGELLA SINGULARIS* HAGL. AND *DISTANTIELLA THEOBROMA* (DIST.),
IN THE GOLD COAST.

PART II. GEOGRAPHICAL AND HABITAT DISTRIBUTION.

By G. WILLIAMS, B.Sc.

The Zoology Department, The University, Reading.

E.M.N.

This is the second part of a paper in which an attempt is made to summarise knowledge of the biology of the two Bryocorine Mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.), which are important pests of cacao in West Africa. Most of the results have been derived from field observations in the Gold Coast, and the sampling methods employed have been described in the first part (Williams, 1953). In the present part, these methods are distinguished by the use of capital initial letters.

The first part considered the factors which determine the distribution and the appearance of Mirid damage in the field, dealing with the problem with the cacao in mind, and making little distinction between the two species. It is the aim in the present part to examine the habits of the Mirids themselves, particularly those in which the two species differ.

Geographical Distribution.

Squire (1947) lists the ranges and food-plants of the various Bryocorine Mirids of West Africa. Both *S. singularis* and *D. theobroma* are essentially Guinean in distribution, so that their ranges overlap considerably. The main difference between them is that *D. theobroma* is not found farther east than the Cameroons whereas the genus *Sahlbergella* appears to reach its maximum complexity in the Congo region. Within the borders of the Gold Coast the ranges of the two species overlap and there is, as yet, no definite evidence that the two are not co-extensive there.

It has been suggested (Squire, 1947) that *D. theobroma* is an invader from the drier north, and is not native to the forest region, the evidence cited including its darker colour, its absence from the south-east of the Guinean region, and the lack of associated parasites. It would certainly appear that *D. theobroma* is the more resistant species to dry conditions as suggested by the following. In the Tafo area its population maximum is a month later in the dry season than that of *S. singularis*; during the Survey of the Cacao-growing Areas it was the only species found in the dry Wenchi area; and it was the only species found in the dry belt immediately to the north of the Gold Coast escarpment, although *S. singularis* was abundant at the same time on the wetter scarp only a few miles to the south. (For places mentioned in the text see fig. 1.)

Whether this is also a reflection of a more northerly distribution cannot be told from this limited collection gathered over a period of only five months, and the remaining evidence is inconclusive. Earlier investigators, covering much of West Africa, judged the species of Mirid present by the age of the cacao attacked, and this cannot be accepted as valid, as will be shown below. Search in the Northern Territories of the Gold Coast, far from the cacao belt, failed to show any sign of *D. theobroma*. This search, made during April 1947, included *Ceiba pentandra* Gaertn., the known alternative host of *D. theobroma*, together with other members of the Bombacaceae, Sterculiaceae, and Tiliaceae, as possible food-plants.

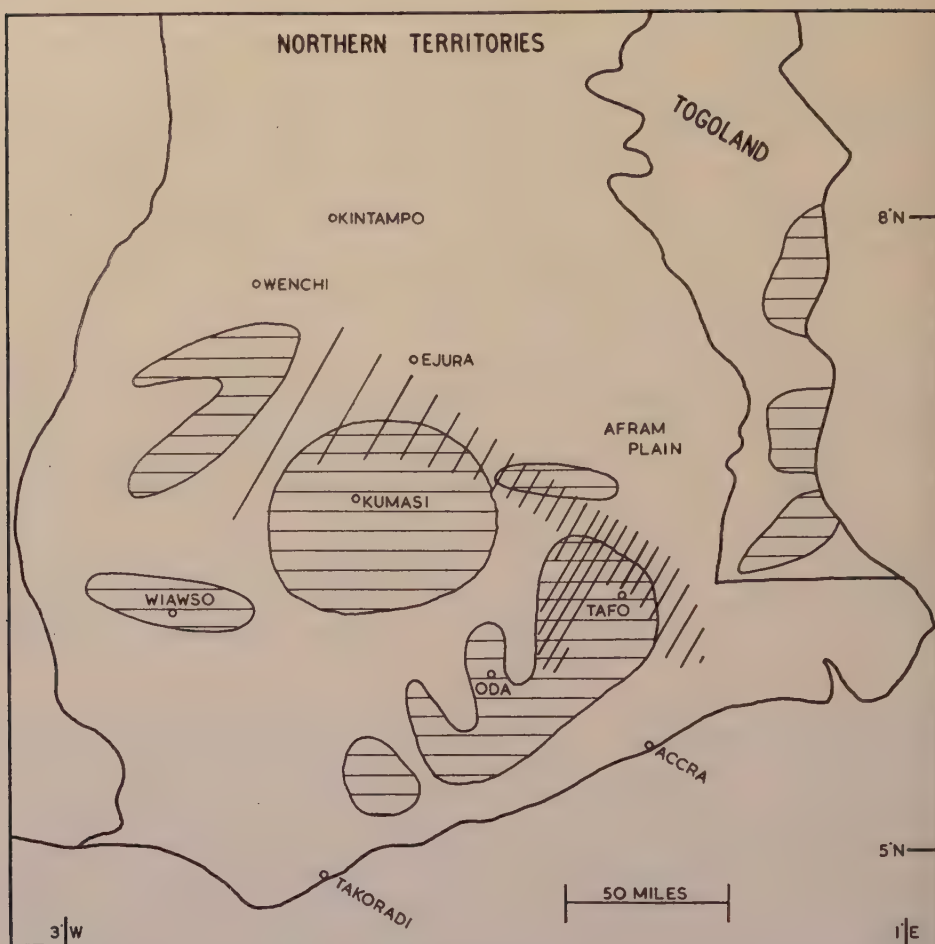


Fig. 1—Sketch map of southern part of the Gold Coast.

Horizontal shading indicates main cacao areas. Oblique shading indicates escarpment.

Within the main cacao belt there has been ample opportunity for the spread of both species on cacao alone, so that interest in differences of distribution centres on the region to the north of the main cacao belt, but not so far north as the Northern Territories. For example, cacao occurs in small patches on the Afram Plain and these small stands are attacked by Mirids, though the species involved is not known. Distribution could be studied here, and in the Wenchi-Ejura-Kintampo triangle. The same areas should show the importance, or lack of importance, of alternative hosts in the spread of the cacao Mirids, a problem difficult to investigate in the main cacao belt. In the Wenchi-Ejura region cacao cultivation is confined to the river valleys, and within these to patches close to villages, or to motor roads. Each little stand is separated from its neighbour by the "fringing forest" that hugs the river valley, or by the dry savannah between valleys. Some of these stands are attacked by Mirids and some, so far, are not. Although the region produces only a small proportion of the total Gold Coast cacao, investigation of the problem of dispersal would be most interesting. Dispersal of the pests by planting material may be ruled out, as the West African farmer uses seed for propagation.

Habitat Preferences.

The only recorded differences between the habits of the two species in the Gold Coast are those of Box (1944) and of Squire (1947), both relating the species of Mirid present to the age of the cacao attacked.

Box made a clear distinction between *S. singularis*, a pest of mature trees, and *D. theobroma*, which confined its attack to seedlings. When *S. singularis* was first observed as a pest of cacao, in the Cameroons in 1902, great damage was caused to trees of all ages, and the same is true today in that country and in others, like the Belgian Congo, where *D. theobroma* is absent. Field observations by the writer, in the Tafo area, showed that seedling cacao did not escape attack by *S. singularis* even in the Gold Coast. From November 1947 to July 1948 inclusive the Routine Collections were closely supervised to ensure that both species of Mirid were searched for with equal care, whatever the age of the tree examined, and Table I shows the total count for that period. Of the total number of *S. singularis* 31 per cent. were taken from seedlings, while 58 per cent. of the total *D. theobroma* were taken from mature trees.

TABLE I.

Distribution of Mirids collected from November 1947 to July 1948 inclusive.

	Number searched	Number of <i>S. singularis</i> found	Number of <i>D. theobroma</i> found
Seedlings ..	34,148	2,553	1,291
Mature trees..	310,920	5,662	1,811

Squire amended the sharp distinction made by Box. He sampled 20 Mirid pockets over a period of seven months and showed, from the total counts, that the proportion of *D. theobroma* to *S. singularis* was inversely related to the age of the cacao sampled. A similar general trend, though not so linear as the relationship found by Squire, can be seen in the results of the Survey of Cacao-growing Areas. During the survey, Mirids were found at 80 of the sampled stands; at 41 of these *D. theobroma* occurred alone, at 30 *S. singularis* occurred alone, and both species were present together at the remaining nine. Ignoring the last nine stations, Table II shows the number of occasions on which cacao in the different age classes was attacked by either species, and from the Table it can be seen that *D. theobroma* was associated, in a general way, with the younger cacao. Using mean values of 3 years, 9, 16, and 25 years for the four age classes the mean age of cacao attacked by *D. theobroma* was 11.8 years \pm 0.99, and by *S. singularis* 15.4 years \pm 1.22, a difference significant at the level $P = 0.05$.

TABLE II.

Age of cacao stands, and frequency of attack by *S. singularis* and *D. theobroma* in the Survey of Cacao-growing Areas.

Age of trees in the stand	Number of times <i>S. singularis</i> present	Number of times <i>D. theobroma</i> present
< 5 years	4	9
6-12 "	3	12
13-20 "	17	17
>20 "	6	3

Despite this population difference, either species may be present on cacao of any age so that the age of the stand attacked cannot be used, as has been done in the past, to determine the species involved. A more reliable guide is provided by the type of damage sustained by the cacao, and this may also account for the apparent association between the species of Mirid and age of the tree. It would appear that *D. theobroma* is virtually restricted to chupons, when feeding on stems, so that fan damage is a symptom of attack by *S. singularis*. During the sampling period in the Observation Plots 19,320 *S. singularis* were counted, and 3,477 *D. theobroma*. *S. singularis* was more evenly spread over the parts of the tree, with 48 per cent. on chupons, 30 per cent. on pods, and 17 per cent. on fans. *D. theobroma* was more markedly confined to chupons, 79 per cent. being found on such stems and 10 per cent. on pods. Only 7 per cent. were on fans and most of these were on the primary fans springing from the jorquette. In each case the remaining 5 per cent. or 4 per cent. were seen on the rough bark of the trunk, or of the thicker branches, and these constituted a hiding or resting population which may be ignored.

This relationship between chupons and *D. theobroma* holds for the Gold Coast in general, for the same 71 stations of the survey used above can be rearranged to show the association between species and the type of damage, as in Table III. Unfortunately, nothing is known of the histories of these stands and previous attacks by the other species may have occurred so that the distinction is not as clear as in the Observation Plots.

TABLE III.

Association between damage sustained by the cacao trees and the species of Mirid present.

Site of Mirid damage	Number of times <i>S. singularis</i> present	Number of times <i>D. theobroma</i> present
Chupons only ..	11	28
Chupons and fan branches ..	19	13

$$\chi^2 = 7.0$$

Damaged trees produce relatively more chupon growth than healthy trees, so *D. theobroma* should be particularly related to badly damaged cacao. (It has been shown by Williams (1953) that the cacao Mirids are primarily invaders of damaged cacao and reference here is not to the damage sustained as a result of attack by Mirids.) Two of the Observation Plots in Mirid pockets were adjacent to two further Observation Plots in healthy cacao, and, of the total number of each species in the four plots, 90 per cent. of *D. theobroma* were observed within the pockets, compared with 72 per cent. of the total count of *S. singularis*. Further, five pockets were sampled by the Observation Plot technique and these can be arranged in order of deterioration, the greater the deterioration the greater the proportion of chupons to fans. When this is done it is found that the proportion of *D. theobroma* to *S. singularis* increases as deterioration increases.

The restriction of *D. theobroma* to chupons and, to a smaller extent, to the primary fans associated with chupons, explains the apparent relationship between this species and young cacao, and explains the frequent exceptions to this rule. The fan-chupon ratio of a seedling is very low and any setback to growth is followed by chupon regeneration. Older cacao, with a developed canopy, has a high fan-chupon ratio and severe damage is needed to induce the production

of chupons. In this way the ideal habitat for *D. theobroma* is more frequently, but not entirely, furnished by young trees.

Competition.

The wider habitat range of *S. singularis* described above is essentially a feeding range only, for oviposition by this species is almost restricted to pods and chupons (Williams, 1953). Fan branches are used for food, particularly by the imagines, but not for oviposition. Consequently, unless chupons are available in excess, there will be competition between the two species for chupons. The same might also be true of pods but these are absent when the total Mirid population is at its highest and the effects of competition presumably at their most intense. The total number of chupons in a large area would not be a limiting factor if the Mirids were uniformly distributed, but the cacao Mirids are localised to small stands which have been made suitable by other agencies (Williams, 1953). Even within such favourable areas the Mirids are not uniformly distributed, but repeated ovipositions are made by both species on a few trees. During the complete period of observation in the Daily Observation Plot all of the 176 trees were visited by Mirids, but at any one time only a small number of trees would be supporting developing nymphs; *e.g.*, in December 1946, when the population of *S. singularis* was at, and the population of *D. theobroma* was near, its maximum the number of infested trees was as shown in Table IV. The number of trees with both species of Mirid present was 23, compared with an expected number of eight had the distribution of the two been random, and the observed number with no Mirids 123 compared with the expected 108. The same concentration of both species on a limited number of trees was also conspicuous at times of very low population. Moreover, Mirids were found, not only on a limited number of trees, but on the same chupons, a single one having been observed with 20 nymphs, representing at least three separate ovipositions.

TABLE IV.

Infestation of cacao trees in the Daily Observation Plot, December 1946.

	Trees with <i>S. singularis</i> nymphs	Trees without <i>S. singularis</i> nymphs	Total
Trees with <i>D. theobroma</i> nymphs	23	18	41
Trees without <i>D. theobroma</i> nymphs	12	123	135
Total	35	141	176

$$\chi^2 = 44.5$$

This concentration would not be important if the chupon was capable of sustaining such attack, nor if the nymphs moved away readily as the stem became less favourable. But neither of these qualifications holds.

Feeding by Mirids is very destructive to the attacked plant, and the devastation of cacao farms is achieved by a population which is very small in comparison with most crop pests. As a specific instance, eight eggs of *D. theobroma* were seen, in the Daily Observation Plot, on a seedling about two feet tall; within 12 days of hatching the nymphs had completely killed the seedling. Such a scale of damage is not only serious to the cacao farmer but adverse to the Mirids, for none of the eight nymphs survived. Three were found dead on the plant, and there was no further sign of the other five despite

a close watch for migrants on nearby trees. Presumably they were taken by predators, or they had died through shortage of food and fallen from the plant into the litter.

In the example cited, migration to a new source of food was precluded as it would have entailed crossing the litter to a new tree. Although chupons of mature trees succumb to attack as quickly as seedlings, migration to a new feeding site should be easier, as more chupons would be available on the same tree. In fact, such migrations are less frequent than would be expected. It was possible to trace the movements of nymphs over infested trees in the Daily Observation Plot, and, although all nymphs changed position some time, most of the movement could be reduced to a general diurnal shift. During the heat of the day nymphs were inactive in the cracks of the bark, at the jorquette or at the base of the chupon, and they spread along the chupon again when feeding was resumed. Even after sheltering on the bark of the main trunk the active nymphs returned to feed on the original chupon, as this was the nearest. A further number of movements was represented by a drift away from fan branches. In this plot there was no canopy so that most of the fans were "primary fans," arising at the jorquette. Such primary fans are similar to chupons, being more succulent than the fine twigs of the canopy, which are the stems generally meant in most of the text when the term fan is used. The unfavourableness of even the primary fans was shown by the migration of the hatched nymph to chupons, and this drift, together with the diurnal shift, accounted for 86 per cent. of the observed movements. The remaining small proportion, 14 per cent., was commensurate only with the death of chupons. During the period prior to death, when the stem was wilting and deteriorating, the Mirids remained, with increased mortality and a prolonged nymphal life.

Mortality in the Daily Observation Plot will be examined in a later paper. The relationship between crowding and length of development could, and should, be investigated experimentally. Although it was an observable phenomenon in the plot it has proved impossible to extract the appropriate data from the records owing to the unrecorded differences between chupons, and to the day-to-day fluctuation in the number of Mirids on a single chupon as a result of new hatchings and mortality. An indirect estimate may be made by comparing the nymphal duration at different population levels, choosing periods sufficiently long to eliminate differences in the number of chupons between periods. For this analysis the year may be conveniently divided into quarters. Considering only *D. theobroma*, which is the more abundant species in the plot, December-January-February is a period of large and increasing numbers with the maximum population in February, the next two quarters have a low average population, and the September-October-November period is intermediate. The complete development cycle, of five nymphal instars from eclosion to the emergence of the adult, was observed for only 41 individuals, for it is difficult to be certain that the first record of a first-instar nymph was made on the day of hatching. If the change from first to second instar is taken as the starting point 158 histories are available, and Table V shows the span of this shorter estimate during the four periods. Examination of the 41 complete histories suggested that the shorter estimate was a reliable measure, for there was a very close correlation between the complete nymphal cycle and the truncated measure ($r = +0.998 \pm 0.057$).

Examination of columns 3 and 4 of the Table shows that the population was greatest in the quarter when the mean nymphal duration was greatest, which is in agreement with the expected effect of crowding suggested by observation. Other factors were not controlled and the quarters chosen also coincide with marked climatic differences, the December to February quarter, for example, being a time of hot, dry days, and cold nights. Two of the pertinent climatic

TABLE V.

Duration of nymphal life (minus first instar) of *D. theobroma* in four periods of the year.

Period	Number of histories	Mean nymphal duration in days	Mean daily population of Mirids in plot	Minimum shade temperature in °F.	Mean R.H. at 3 p.m.
xii, i, ii	66	18.5±0.30	151	59.3	55.1
iii, iv, v	46	17.0±0.23	37	63.5	63.1
vi, vii, viii	24	16.4±0.35	37	67.8	75.1
ix, x, xi	22	17.2±0.31	50	66.3	72.5

factors are given in the last two columns of Table V, *viz.*, the mean minimum temperature during the period, and the mean relative humidity at 3.00 p.m. as recorded from instruments in a Stevenson screen about a mile away from the plot. The longer nymph span coincides with the period of low relative humidity and low minimum temperature so that the direct effect of crowding cannot be proved from these field records and needs experimental confirmation. In one respect the climatic differences between the periods reinforce the effects of crowding, the drier days of the December–February quarter accelerating the wilting of the attacked chupons.

The same analysis is true for *S. singularis*, but is not presented here as the numbers are smaller, and no amendment of the results derived from consideration of *D. theobroma* is needed.

Observations suggest, therefore, that only a small proportion of chupons in an area is available to Mirids, and that these chupons are rapidly spoiled as a result of the destructive nature of Mirid feeding, coupled with the sedentary habits of the nymphs. Interspecific competition must be an important factor in Mirid ecology, and interest centres on the relationship, in the field, between these two closely related species which were included in the same genus by Distant (1909) before being placed in distinct genera by China (1944).

The spread, and indeed the continued existence, of either species depends upon the successful invasion of areas which have become suitable for Mirids by the action of other agencies (Williams, 1953). As an invader, the balance of advantage would seem to lie with *S. singularis* for the ability of the adult to feed on fans would enable it to live temporarily in areas not suitable for development, and to extend its range. Moreover, laboratory rearings suggest that *S. singularis* has a greater fecundity than *D. theobroma*, estimates from 38 females of each species giving the mean number of eggs produced as 51.2 ± 6.34 for *S. singularis* and 32.3 ± 4.18 for *D. theobroma*.

Despite these differences *D. theobroma* seems not only to be holding its own in the Gold Coast but also to be increasingly important. An indication of the possible advantage possessed by *D. theobroma* is given by the same laboratory rearings that were used above. Although the total number of eggs per female was less, the rate at which they were produced was higher for *D. theobroma* than for *S. singularis*, the mean number of eggs produced per day, for days when oviposition took place, being respectively 5.5 ± 0.33 compared with 4.2 ± 0.17 . The quicker turn-over by *D. theobroma* would be aided by its shorter life-history, for Voelcker (1945) gives the mean time from oviposition to egg-laying adult as 42.7 days for *D. theobroma* compared with 47.9 days for *S. singularis*. The rearing conditions were artificial as the insects were disturbed each day when transferred to new food material. Such laboratory results need checking against field conditions, but direct estimates of fecundity and longevity in the Observation Plots were not possible. The adults were not confined in any way, and, as there was continual migration to and from the plots the number of nymphs

emerging could not be related directly to a definite number of females. The field data can be used to furnish indirect evidence which supports the laboratory findings.

In the Daily Observation Plot the spot at which a female Mirid was seen was subsequently searched for eggs. The most seen in a "clutch," produced by rapid successive ovipositions, has been four for *S. singularis* while clutch sizes of up to 12 eggs have been observed for *D. theobroma*. The eggs of both species are difficult to detect as they are inserted beneath the bark of the stem with only a pair of slender filaments protruding, but eggs of *S. singularis* are the more easily overlooked. The egg counts cannot be considered conclusive but an estimate of clutch size can also be made from the numbers in the groups of first-instar nymphs, for the mortality in the egg is low, and the hatching nymphs remain together as a definite group for some time. Table VI summarises for each species the frequency distribution of the size of first-instar groups, and the mean number was higher for *D. theobroma* (2.9) than for *S. singularis* (2.0), a difference which proves to be significant when the original counts are tested by the estimation of χ^2 . The difference was not attributable to differential mortality for survival into the second instar of nymphs watched from the time of hatching was 85 per cent. for *S. singularis* and 80 per cent. for *D. theobroma*.

TABLE VI.
Percentage distribution of the size of groups of first-instar nymphs.

Species	Number of groups	Number in group						
		1	2	3	4	5	6	>6
<i>S. singularis</i> ..	192	48.4	27.1	12.0	7.8	1.0	1.6	2.1
<i>D. theobroma</i> ..	686	29.7	27.3	15.6	11.1	7.0	3.3	6.0

The greater clutch size of *D. theobroma* is associated with a shorter stay on the tree. In the Daily Observation Plot it was assumed that two consecutive records of an adult of the same sex of the same species on the same stem implied that the same individual was being recorded, and marked adults have been observed to remain on the same stem for eight days. Table VII summarises the observed times spent on a tree, the maximum noted being 15 days for one female

TABLE VII.
Length of time adult Mirids spend on a tree.

Number of whole days spent on the tree	<i>S. singularis</i>				<i>D. theobroma</i>			
	Female		Male		Female		Male	
	Develop- ing	Migrat- ing	Develop- ing	Migrat- ing	Develop- ing	Migrat- ing	Develop- ing	Migrat- ing
0	48	72	19	13	171	152	41	37
1	41	77	14	15	116	140	37	30
2	20	37	10	6	73	70	15	8
3	16	19	2	5	30	24	6	4
4	10	17	4	6	16	14	2	3
5	4	8	2	3	9	12	2	2
6	5	2	1	2	3	4	1	1
>6	2	4	0	1	4	4	2	0
Total	146	236	52	51	422	420	106	85

of each species. The pertinent value here would be the time spent in one place by a mature female but it is impossible to distinguish externally between mature and immature adults. However, the adults in the plot may be divided into two classes: those which have developed from nymphs already present on the tree, and those which arrived by migration. All in the first class, but only a proportion of those in the second, would have been immature, so that the behaviour of the fertile female may be inferred by comparing the two classes. Hence the division into two methods of arrival on the tree in Table VII. An analysis of variance was performed by the method of Yates (1934) and the only significant differences were those between species. The duration of the visit did not differ between sexes of the same species, nor between those developing on the tree and those arrived by migration. The mean lengths of stay, together with the appropriate standard errors, are given in Table VIII.

TABLE VIII.

Mean length of stay (in days) with standard errors. The numbers in brackets are means corrected for unobserved visits of less than 24 hours.

	Female	Male
<i>S. singularis</i>	1.59 ± 0.096 (1.21)	1.69 ± 0.178 (1.29)
<i>D. theobroma</i>	1.24 ± 0.053 (0.90)	1.10 ± 0.103 (0.78)

As the records in the plot were made once a day, all Mirids remaining longer than 24 hours were recorded, but some proportion of those staying less than 24 hours must have been missed. Examination of the frequency distribution suggested that a smooth curve could be obtained for both species by doubling the number observed to stay less than 24 hours. Differences between the two species are therefore unlikely in this unobservable part of the curve, and the estimate of the mean stay obtained by using the doubled value is probably a more realistic one and so it has been given, in brackets, in Table VIII. The larger clutch size of *D. theobroma* was therefore associated with a visit of less than one day and the smaller clutch size of *S. singularis* with a visit of more than one day. By using the numbers in a group of first instars any errors due to laying a larger number of small clutches would be cancelled out as the hatching nymphs would be included as a single group.

Although the values obtained in the laboratory and in the field differ, the relationship between the two species in the rates of egg-laying remain the same and one may conclude that *D. theobroma* has a more rapid build-up in localised areas. This is in agreement with general field experience for, where *D. theobroma* does exist it generally occurs in large numbers, as many as 25 nymphs having been seen by the writer on a single seedling, about three feet high, which had only reached the stage of jorquetting once.

D. theobroma in Nigeria.

Nigeria is not the easternmost limit of the distribution of *D. theobroma*, but, whereas *S. singularis* is an important pest in that country, *D. theobroma* is reported not to be. It may be of interest to examine the two features which favour *D. theobroma* in the Gold Coast; an abundance of chupons, and a high rate of egg production.

In Nigeria, cacao is generally cultivated without the provision of overhead shade by other trees, and under such conditions damage takes the form of a diffuse stagheadedness (Williams, 1953). Proliferation, induced by damage, is

by the production of new canopy fans, and few chupons are produced. Moreover, the Nigerian farmer removes any chupons which spring from the base of the trunk unless the old trunk is dying, when a new chupon is allowed to grow and utilise the old root system.

At Owena, in the Ondo province, Mirids were reared by methods similar to those used at Tafo. The relationship between the rates of egg-laying appeared to be reversed, the mean number produced per day of active oviposition being 4.6 ± 0.10 for *S. singularis*, and 4.0 ± 0.14 for *D. theobroma*. As emphasised above, the laboratory conditions were artificial so that too much reliance must not be placed on these estimates, but this change is a feature of Mirid biology which should be investigated further. A Daily Observation Plot was established at Owena but the incidence of *D. theobroma* was too low to allow the same comparisons to be made there as were made in Tafo.

The interaction of the reduced amount of chupon material and a rate of oviposition lower than that of *S. singularis* may help to explain the relative unimportance of *D. theobroma* in Nigeria.

Summary.

The geographical ranges of *Sahlbergella singularis* and *Distantiella theobroma* overlap, and the Gold Coast lies in the zone common to both. The respective northern limits remain to be determined, and should be investigated to the north of the main cacao belt, from Kintampo to the Afram plain.

The same area should also yield information on the possible importance of alternative hosts in the dispersal of cacao Mirids, as the small stands are well separated from each other.

Earlier records, that *D. theobroma* is confined to seedlings and *S. singularis* to mature trees, are erroneous. *D. theobroma* is found on pods and, particularly, chupons. The feeding range of *S. singularis* includes fan branches in addition, but breeding is confined to chupons and pods.

Observations suggest that there is competition for breeding sites on chupons. Of the many chupons in an area only a small number is available to Mirids, and the proportion of nymphs reaching maturity is limited by their sedentary habit and the destructiveness of their feeding.

Co-existence of the two species is possible by their slightly different habits. *S. singularis* produces more eggs, but is more diffusely distributed. *D. theobroma* is virtually restricted to chupons, but can quickly build up a large population in favourable areas by its greater rate of egg production.

In Nigeria, the rate of egg production by *D. theobroma* is less than that by *S. singularis*, and this, together with the sparsity of chupons, may account for the unimportance of *D. theobroma* in that country.

Acknowledgements.

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References.

- Box, H. E. (1944). The *Sahlbergella* menace to Gold Coast cacao.—Memor. cent. Cocoa Res. Sta. no. 9, 8 pp.
- CHINA, W. E. (1944). New and little known West African Miridae (Capsidae) (Hemiptera Heteroptera).—Bull. ent. Res., **35**, pp. 171–191.

- DISTANT, W. L. (1909). Contributions to a knowledge of Ethiopian economic entomology.—*Entomologist*, **42**, pp. 252-253.
- SQUIRE, F. A. (1947). On the economic importance of the Capsidae in the Guinean Region.—*Rev. Ent.*, **18**, pp. 219-247.
- VOELCKER, O. J. (1945). West African Cacao Research Institute. Annual Report, April, 1944, to March, 1945.—36 pp.
- WILLIAMS, G. (1953). Field observations on the cacao Mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.), in the Gold Coast. Part I. Mirid damage.—*Bull. ent. Res.*, **44**, pp. 101-119.
- YATES, F. (1943). The analysis of multiple classifications with unequal numbers in the different classes.—*J. Amer. statist. Ass.*, March 1934, pp. 55-60.
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THE SUSCEPTIBILITY TO DDT DUST OF COLEOPTERA INFESTING STORED PRODUCTS.

By E. A. PARKIN.

Pest Infestation Laboratory, Slough, Bucks.

Laboratory evaluation of insecticides tends to be restricted to tests with a few species of insects which can be bred conveniently under standardised conditions. In practice, however, infestation of stored products frequently involves a mixed population of insects, among which one or two species are considered of primary and the remainder of secondary importance. Control measures must be directed particularly against the species of primary importance, but it is clearly advantageous that information should be available on the susceptibility to different insecticides of a wide range of species, so that the total effect of a recommended treatment in a warehouse, mill, etc., may be the more accurately forecast. Otherwise there is a risk that control of the primary pest will lead merely to its place being taken by a former secondary species of higher resistance.

To test all the combinations of the common species of stored product insects, their different stages of development, the insecticides suitable for use in warehouses, etc., their different formulations, and methods of application would require a large organisation and many years of work. Such data, however, are badly needed, and as a first contribution towards filling this gap in our knowledge an investigation has been undertaken to determine the susceptibility to a 5 per cent. DDT dust of a number of the commoner species of stored-product Coleoptera in the larval and adult stages. The experiments have been carried out at intervals over a period of more than two years, as sufficient numbers of the desired stage of each species became available.

Experimental Technique.

The object of the investigation was to divide the species of insects used into broad groups representing different levels of susceptibility to the insecticide, and very careful control of factors known to be likely to introduce some variability into the results was not necessary. Nevertheless, such precautions as could easily be taken were maintained, *e.g.*, after counting into groups, the insects were starved and rested overnight before the start of their exposure to the dust, in order to obtain greater homogeneity of the results (Parkin & Green, 1943). The experimental technique was kept simple by exposing the insects to an excess of dust. It was also convenient to use the period of exposure as the variable dosage factor; but the results so obtained are also closely related to the observations likely to be made under practical conditions when a fixed concentration of insecticide is used against a mixture of insect species.

Insects of mixed ages were taken from thriving cultures bred at 25°C. and 70 per cent. R.H. in the insectaries of the Laboratory. The commoner stored product beetles and their larvae were selected for test, but several uncommon species of PTINIDAE were available and were included to increase the representation of this family. Two batches of 50 insects were exposed to DDT dust and a third kept in an empty dish as control; in most experiments a batch was also exposed in talc. Large insects, *e.g.*, *Tenebrio molitor* L., were exposed in batches of 25, and the highly cannibalistic larvae of *Tenebroides mauritanicus* (L.) had to be exposed individually in 3 × 1 in. glass tubes.

The insecticidal dust was a commercial preparation containing 5 per cent. by weight of crude DDT in kaolin: the crude DDT contained 77 per cent. by weight of the p,p' compound. Approximately 2 gm. of the dust were distributed as uniformly as possible over the bottom of a 9 cm. petri dish by tapping it from a small glass jar fitted with a metal gauze insert in the lid. The dust was allowed to equilibrate for 2-3 days at 25°C. and 70 per cent. R.H. before the insects were added. Lids were not put on the petri dishes, except to confine some of the more active species during the early part of the exposure period. The numbers of insects dead were counted at intervals, usually determined by preliminary test as suitable for the particular species and stage under investigation. An insect was considered dead if it neither moved spontaneously nor responded to slight pressure. Most exposures were stopped at or before the 28th day because the majority of species had suffered a heavy mortality from starvation alone in this period.

Results.

The mean percentage mortalities after the different periods of exposure were calculated from the data for the replicates and corrected for mortality during the corresponding periods among the insects kept in the empty dishes: on the whole, the agreement between replicates was good. The resulting figures were transformed to probits and plotted against the logarithm of the exposure period in hours. Provisional regression lines were carefully fitted by eye, using a single straight line if possible. A set of points which appeared to be best fitted by a curvilinear regression line could be represented with little error by two straight lines of different slopes. Some typical data are illustrated in fig. 1. The periods of exposure required for mortalities of 50 and 95 per cent. were determined from the provisional regression lines and are listed in Table I.

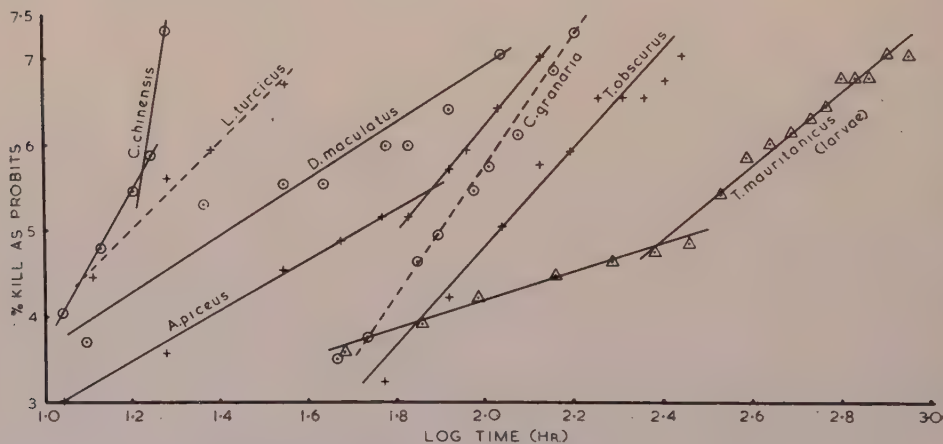


Fig. 1.—Examples of regression lines obtained from exposure of insects to a 5 per cent. DDT dust.

Analysis of the results from exposure of adult insects was fairly straightforward, as the percentages of deaths among the controls were relatively low. With larvae of some species, however, the death rate of the controls was nearly as high as that of the larvae exposed to DDT dust and corrections for control mortality could not reasonably be applied. Under these circumstances, the figures marked with an

asterisk for LT50 and LT95 give little indication of the susceptibility of the insects to the DDT dust owing to the major influence on the death rate of starvation or abnormal conditions of confinement. A considerable proportion of the larvae of three species pupated before even the LT50 had been reached and observations were therefore stopped.

TABLE I.

Hours of exposure to 5 per cent. DDT dust required to cause 50 per cent. (LT 50) and 95 per cent. (LT95) mortality of different species and stages of the Coleoptera of stored products.

Family	Species	Adults		Larvae	
		LT 50 hr.	LT 95 hr.	LT 50 hr.	LT 95 hr.
Anobiidae ..	<i>Lasioderma serricorne</i> (F.) ..	51	88	>660 (4%) †	Pupated 125*
	<i>Stegobium paniceum</i> (L.) ..	38	57		
Anthribidae ..	<i>Araecerus fasciculatus</i> (Deg.) ..	18	21		
Bostrychidae	<i>Rhizopertha dominica</i> (F.) ..	186	460*		
Bruchidae ..	<i>Acanthoscelides obtectus</i> Say ..	18	25		
	<i>Callosobruchus chinensis</i> (L.) ..	15	18		
	<i>Caryedon fuscus</i> (Goeze) ..	53	78		
Cucujidae ..	<i>Laemophloeus ferrugineus</i> (Steph.)	20	26	48	114
	„ <i>minutus</i> (Ol.) ..	18	21	30	220
	„ <i>turcicus</i> Grouv. ..	16	33	93	124
	<i>Oryzaephilus surinamensis</i> (L.) ..	33	54	19	43
	„ <i>mercator</i> Fauv. ..	23	36	25	55
Curculionidae	<i>Calandra granaria</i> (L.) ..	80	135		
	„ <i>oryzae</i> (L.) ..	76	140		
Dermestidae	<i>Attagenus piceus</i> (Ol.) ..	52	115	266	750
	<i>Dermestes lardarius</i> (L.) ..	47	117	420*	>480 (52%)*
	„ <i>maculatus</i> Deg. ..	26	83	89	339
	<i>Trogoderma granarium</i> Everts ..	37	95	>1200 (24%)	
	„ <i>versicolor</i> (Creutz) ..	25	51		
Nitidulidae ..	<i>Carpophilus hemipterus</i> (L.) ..	26	46	Pupated	
	„ <i>ligneus</i> Murray ..	47	115	>133 (19%)	Pupated
Ostomatidae	<i>Tenebroides mauritanicus</i> (L.) ..			275	660
Ptinidae ..	<i>Eurostus hilleri</i> (Rttr.) ..	299	457		
	<i>Gibbium psyllodes</i> (Czenp.) ..	845	1520		
	<i>Ptinus sexpunctatus</i> Panz. ..	114	228		
	„ <i>tectus</i> Boield. ..	251	352	400*	>700 (52%)*
	<i>Stethomezium squamosum</i> Hinton	407	871		
	<i>Trigonogenius globulus</i> Sol. ..	246	471		
Tenebrionidae	<i>Caenocorse ratzeburgi</i> (Wissm.) ..	66	90	39	69
	<i>Gnathocerus cornutus</i> (F.) ..	25	45	33	55
	<i>Latheticus oryzae</i> Waterh. ..	43	63	43	140
	<i>Tenebrio molitor</i> L. ..	69	105	143	513
	„ <i>obscurus</i> F. ..	106	211	305	498
	<i>Tribolium castaneum</i> (Hbst.) ..	44	69	62	171
	„ <i>confusum</i> Duv. ..	65	105	81	191
	„ <i>destructor</i> Uyttenb. ..	364	463	400	>695 (88%)*

* Control deaths excessive: kills not corrected.

† Figures in brackets are the percentage deaths at the given period of exposure.

For practical purposes, the results are probably most usefully expressed by dividing the species into groups of similar resistance based on the time required for the 5 per cent. DDT dust to effect 95 per cent. kill.

A. Adults with LT95 less than 2 days.

<i>Callosobruchus chinensis</i>	<i>Laemophloeus turcicus</i>
<i>Araecerus fasciculatus</i>	<i>Oryzaephilus mercator</i>
<i>Laemophloeus minutus</i>	<i>Gnathocerus cornutus</i>
<i>Acanthoscelides obtectus</i>	<i>Carpophilus hemipterus</i>
<i>Laemophloeus ferrugineus</i>	

B. Adults with LT95 between 2 and 4 days.

<i>Trogoderma versicolor</i>	<i>Caryedon fuscus</i>
<i>Oryzaephilus surinamensis</i>	<i>Dermestes maculatus</i>
<i>Stegobium paniceum</i>	<i>Lasioderma serricorne</i>
<i>Latheticus oryzae</i>	<i>Caenocorse ratzeburgi</i>
<i>Tribolium castaneum</i>	<i>Trogoderma granarium</i>

C. Adults with LT95 between 4 and 8 days.

<i>Tribolium confusum</i>	<i>Dermestes lardarius</i>
<i>Tenebrio molitor</i>	<i>Calandra granaria</i>
<i>Carpophilus ligneus</i>	<i>Calandra oryzae</i>
<i>Attagenus piceus</i>	

D. Adults with LT95 between 8 and 16 days.

<i>Tenebrio obscurus</i>	<i>Ptinus tectus</i>
<i>Ptinus se xpunctatus</i>	

E. Adults with LT95 more than 16 days.

<i>Eurostus hilleri</i>	<i>Trigonogenius globulus</i>
<i>Rhizopertha dominica</i>	<i>Stethomezium squamosum</i>
<i>Tribolium destructor</i>	<i>Gibbium psylloides</i>

A similar general classification can be made of the larvae.

A. Larvae with LT95 less than 2 days.

<i>Oryzaephilus surinamensis</i>

B. Larvae with LT95 between 2 and 4 days.

<i>Gnathocerus cornutus</i>	<i>Caenocorse ratzeburgi</i>
<i>Oryzaephilus mercator</i>	

C. Larvae with LT95 between 4 and 8 days.

<i>Laemophloeus ferrugineus</i>	<i>Latheticus oryzae</i>
<i>Laemophloeus turcicus</i>	<i>Tribolium castaneum</i>
<i>Stegobium paniceum</i>	<i>Tribolium confusum</i>

D. Larvae with LT95 between 8 and 16 days.

<i>Laemophloeus minutus</i>	<i>Dermestes maculatus</i>
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E. Larvae with LT95 more than 16 days.

<i>Tenebrio obscurus</i>	<i>Attagenus piceus</i>
<i>Tenebrio molitor</i>	<i>Ptinus tectus</i>
<i>Dermestes lardarius</i>	<i>Lasioderma serricorne</i>
<i>Tenebroides mauritanicus</i>	<i>Trogoderma granarium</i>
<i>Tribolium destructor</i>	

Discussion.

The most striking feature shown by these results is the wide range of resistance among the species included. Although 36 species of beetles were used, they belonged to 11 families of which only four were represented by three or more

genera. Among the adults of these families, the Ptinids were all relatively resistant, the Tenebrionids varied from very susceptible to very resistant, the Dermestids were susceptible, and the Bruchids were very susceptible. Large differences were sometimes recorded between genera in the same family, e.g., adults of *Ptinus* and *Gibbium*; or between species of the same genus, e.g., adults and larvae of *Tribolium castaneum* and *T. destructor*.

In general, the larvae tended to be more resistant than the corresponding adults and this was particularly so with *Lasioderma serricorne* and the Dermestids. Whereas the adults of *Trogoderma granarium* were relatively quickly killed by the DDT dust, the larvae were very highly resistant to it.

The larvae used in the tests were one-half to nearly fully grown and the following 11 species produced at least one pupa 24 hours or more after first contact with the powder: *Tribolium confusum*, *T. destructor*, *Oryzaephilus surinamensis*, *O. mercator*, *Dermestes lardarius*, *D. maculatus*, *Tenebrio molitor*, *T. obscurus*, *Carpophilus hemipterus*, *C. ligneus*, and *Lasioderma serricorne*. For example, in a test with *T. destructor* a larva pupated between the 12th and 14th days of exposure, a second between the 23rd and 24th days, and a third between the 24th and 26th days. On the other hand, *Trogoderma granarium* was kept for the full 28 days in the powder with a relatively low kill and no pupation. One or more adults of *D. maculatus*, *T. molitor*, *T. obscurus*, and *C. hemipterus* emerged from pupae formed during the tests, but observations were not completed because the majority of tests were terminated on the 28th day.

It does not seem possible to draw from the results any definite conclusions linking resistance with size or activity, or with the presence of scales or setae. At most, there was a tendency for the more active insects to be the more susceptible.

The results for the beetles were examined statistically to determine whether there was a significant correlation between the slopes of the various probit regression lines (determined from the LT50 and LT95 values) and the corresponding log-LT50 values, but the correlation coefficient ($r = -0.235$ for 33 df) was not significant (if $P = 0.05$, $r = -0.349$ for 30 df). In this analysis, no weighting adjustment was made for precision of the individual observations. There is therefore no evidence that the species which are slow in dying have a wider distribution of the individual lethal-exposure times.

Finally, this investigation has been confined to Coleoptera since tests with larvae of Lepidoptera (*Ephestia* spp., *Plodia* sp.) failed to give interpretable results. The lepidopterous larvae seemed to be moderately susceptible to the 5 per cent. DDT powder but progressed only very slowly to a state which could definitely be defined as "death". Moreover, many spun cases on the lids of the dishes out of contact with the insecticide and others webbed over the surface of the powder, thus greatly reducing their contact with it.

Information in the literature upon the resistance to DDT dusts of insect pests of stored foodstuffs is very scattered and fragmentary. Most authors appear to have been concerned only incidentally with determining relative resistance and then have compared only two or three species. The most extensive work is that of Zinkernagel, Gasser and Domenjoz (1946) who studied 11 species of beetles and three of moths. The results reported by different workers are often contradictory. For example, Swingle and Mayer (1944) and Zinkernagel and others (1946) found *Tribolium* spp. more resistant than *Calandra* spp. whereas Cherian and Rao (1945) found the reverse: moreover, Zinkernagel and others (1946) state that *Rhizopertha* was more resistant than *Calandra*, yet Aboim (1948) reports the opposite. Different experimental techniques and especially different ages of the test insects may well explain these differences in results. Nevertheless, a few conclusions of interest arise from the literature. Bean weevils (Bruchids) have been found to be very susceptible to DDT (Lepage & Giannotti, 1944; Swingle & Mayer, 1944; Aboim, 1948) as also are adult moths such as *Sitotroga*, *Ephestia*, and *Tinaea* (Nasir, 1946;

Zinkernagel & others, 1946; Aboim, 1948). Larvae are more resistant than the corresponding beetles or moths (Cotton & others, 1945; Nasir, 1946; Zinkernagel & others, 1946). Nasir points out the very high level of resistance of larvae of *Trogoderma granarium*. These results are in general agreement with those of the present more extensive work. In conclusion, it should be pointed out that insects which have shown some resistance to a 5 per cent. DDT dust in the present work may well be controlled satisfactorily in practice by dusts and other formulations having higher DDT contents.

Summary.

The resistance to a 5 per cent. DDT powder of 36 species of adult and larval Coleoptera which infest stored products has been determined. The species are grouped according to the time required to effect 95 per cent. kill. In the data for adults, the slopes of the probit regression lines were not significantly correlated with the log-times for 50 per cent. kill. No obvious connection could be seen between the degree of resistance to the insecticide and size, activity, hairiness, etc., but larvae tended to be much more resistant than the corresponding adults.

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References.

- ABOIM, A. N. (1948). Action du " DDT " contre plusieurs ravageurs des denrées emmagasinées.—Bull. Soc. portug. Sci. nat., **16**, pp. 75–80.
- CHERIAN, M. C. & RAO, P. R. N. (1945). Trials with DDT and 666 against pests of stored grains.—Indian Fmg, **6**, pp. 572–573.
- COTTON, R. T., FRANKENFELD, J. C., WALKDEN, H. H. & SCHWITZGEBEL, R. B. (1945). Tests of DDT against insect pests of stored seed, grain and milled cereal products.—U.S. Dep. Agric., Bur. Ent., E-641, 7 pp.
- LEPAGE, H. S. & GIANNOTTI, O. (1944). Experiencias com o DDT.—Biológico, **10**, pp. 353–366.
- NASIR, M. M. (1946). DDT, 666 and insect pests of stored grains.—Curr. Sci., **15**, pp. 98–99.
- PARKIN, E. A. & GREEN, A. A. (1943). A film technique for the biological evaluation of pyrethrum-in-oil insecticides for use against stored product insects in warehouses.—Ann. appl. Biol., **30**, pp. 279–292.
- SWINGLE, M. C. & MAYER, E. L. (1944). Laboratory tests of DDT against various insect pests.—J. econ. Ent., **37**, pp. 141–142.
- ZINKERNAGEL, R., GASSER, R. & DOMENJOZ, R. (1946). Über Getreidekonservierung. II. Insektenbekämpfung mit insektiziden Stäubemitteln.—Mitt. schweiz. ent. Ges., **19**, pp. 653–691.

THE RATE OF DIGESTION OF BLOOD MEALS OF VARIOUS HAEMATOPHAGOUS ARTHROPODS AS DETERMINED BY THE PRECIPITIN TEST.

By B. WEITZ *

Lister Institute of Preventive Medicine, Elstree, Herts.

and

P. A. BUXTON

London School of Hygiene and Tropical Medicine.

Meals of mammalian blood in insects have frequently been identified by the precipitin test. The successful identification depends not only on the quality of antisera used, but also on the nature and the quantity of blood serum proteins available in the gut of the arthropod at the time of capture. These conditions are largely dependent on the original volume of the blood meal, and apart from the evident differences in size of various species of blood suckers, other factors such as their particular method of feeding, the tolerance of the host to the infliction of bites, and the natural palatability of the host to the Arthropods which feed on them, will influence the size of the original blood meal.

The rate of digestion of the serum proteins of a given volume of blood meal will in turn vary considerably in different blood suckers and in different environmental conditions, as for instance the effect temperature has on the rate of digestion in *Aedes* species which West and Eligh (1952) have recently shown.

It is therefore of interest to investigate the rate of digestion in various blood-sucking Arthropods fed on known hosts and kept under standard laboratory conditions after feeding and to compare such observations with the rate of digestion which occurs under natural conditions, when possible.

Materials.

Ten different species of blood suckers were used:

1. *Aedes aegypti* (L.). Adult females from a laboratory culture were used when about 1½ weeks old: they had not previously been fed on blood and were allowed to feed on man.
2. *Anopheles maculipennis atroparvus* van Thiel. Adult females 3 to 7 days old not previously fed on blood were fed on man.
3. *Anopheles aquasalis* Curry. These were wild insects which were allowed to enter a large cage in which an ox was tethered. The cage was left open for two hours (from 18.00 hrs. to 20.00 hrs.) and the mosquitos were caught inside the cage the following morning at 06.00 hrs. The first 50 specimens were squashed at that time, representing 10-12 hour feeds and the remainder were caged separately and killed in batches after the periods shown in the Table.
4. *Culex molestus* Forsk. Adult females of various ages, which had been fed on sugar and raisins previous to the experiments, were fed on man.
5. *Culicoides nubeculosus* (Mg.). Laboratory strains fed on man.
6. *Cimex lectularius* (L.). Mixed male and female laboratory specimens, of various ages, were fed on rabbit and then starved for at least 3 weeks before being fed on man.

* This work was done as part of a programme recommended by the Colonial Medical Research Committee to the Colonial Office, and partially financed with funds under the Colonial Development and Welfare Act.

7. *Bdellonyssus bacoti* (Hirst). Adult female mites were used which had previously been fed on baby rodents; their last feed before the experimental feed on man was on a baby mouse four days previously.
8. *Ornithodoros moubata* (Murr.). These ticks, of various stages, had been fed on rabbit until 3 weeks before the experiment. Some ticks were fed experimentally on Rhesus monkey, others were fed on domestic pig and some on fowl.
9. *Glossina morsitans* Westw. Laboratory-bred flies were given their first meal on man, ox, sheep and goat until gorged.
10. *Glossina swynnertoni* Aust. were collected in a selected area in the field and the "hunger stage" of each individual was established by their external appearances at the time. The blood meals were examined for the presence of nucleated corpuscles and only those which had fed on mammals were used for these experiments.

Experimental Methods.

The laboratory-fed arthropods (*i.e.*, all except numbers 3 and 10) were examined after feeding on their experimental hosts and only gorged specimens were retained for the experiment. At stated intervals a proportion of the specimens were killed and the blood meal was smeared on filter paper and allowed to dry. During the whole period of digestion the laboratory-fed insects were kept in cages at 25°C. and 80 per cent. relative humidity.

Precipitin Tests.

The piece of filter paper containing the whole blood meal was put into 0.5 ml. to 0.25 ml. of normal saline according to the condition of the feed, and kept at 4-6°C. overnight to allow the serum proteins to dissolve. Small quantities of these extracts were layered over the undiluted antiserum in narrow tubes (2-3 mm. bore) and incubated for 2 hours at room temperature. The tubes were then examined by means of indirect illumination against a dark background. Precipitates in the form of a "whitish ring" at the interface of the blood meal extract and the antiserum were recorded as positive. Tubes which showed no such precipitate were recorded as negative.

The antisera, which had been prepared by inoculation of alum-precipitated serum into rabbits and by subsequent absorption of non-specific antibodies as described by Weitz (1952), were tested on each occasion for specificity and for the concentration at which the corresponding antigen showed a positive result. As these antisera were kept in a freeze dried state, no significant variation of the titres were observed from test to test.

Results and Discussion.

The Tables show the numbers of blood meals tested at each stage and the period during which they were positive by specific precipitin tests.

Under the conditions of the experiment it appears that mosquitos and midges almost invariably yield positive results up to 12-16 hours and a high proportion up to 24 hours, after which the proportion of positive results fell rapidly. The longest interval after which feeds were positive in this group of insects was 3 days with *Aedes aegypti* and *Anopheles maculipennis atroparvus*. Such results agree with the early observations of Bull and King (1923) who estimated that *A. quadrimaculatus* Say showed positive feeds up to a period of 24 hours. West and Eligh (1952) found that *Aedes aegypti* showed 100 per cent. positive results after 1 day and 8.5 per cent. positives at 2 days, when the mosquitos were kept at 27°C. during the period of digestion.

TABLE I.
Results of precipitin tests on blood meals of arthropods after varying periods of digestion.

Species of arthropod	Host	Number and percentage of positive meals per group tested after :										
		Hours						Days				
		3	6	16	20	24	30	40	2	3	4	5
<i>Culex molestus</i> ..	Man					11/11 100				0/5 0		0/5 0
<i>Culicoides nubeculosus</i>	Man					4/5 80				0/5 0		
(*) <i>Anopheles aquasalis</i> ..	Ox			45/48 95	13/50 26		2/50 4	0/48 0		0/48 0		
<i>Anopheles maculipennis</i> <i>atroparvus</i> ..	Man			10/10 100						1/11 9	0/10 0	0/2 0
<i>Aedes aegypti</i> ..	Man			10/10 100						3/11 27	0/10 0	0/19 0
<i>Glossina morsitans</i> ..	Man	20/20 100				20/20 100			20/20 100	19/19 100		
	Ox	20/20 100				20/20 100			20/20 100	20/20 100		
	Sheep	20/20 100				20/20 100			20/20 100	15/20 75		
	Goat	20/20 100				20/20 100			20/20 100	18/20 90		
(†) <i>Glossina swynnertoni</i> ..	Mammal				I 2/2 100	II 5/9 55				III 7/25 28	IV 1/44 7	
<i>Bdellonyssus bacoti</i> ..	Man		5/5 100			9/10 90				0/3 0		0/10 0

* Naturally occurring mosquitos.

† Naturally occurring flies.—Hunger stages are indicated by Roman numerals I, II, III, IV, where they correspond to the estimated period of digestion.

TABLE II.

Species of arthropod	Host	Number and percentage of positive meals per group tested after varying periods of days												
		1	5	10	20	30	35	50	65	90	120	150	180	210
<i>Cimex lectularius</i>	Man.		10/10 100	29/30 97	4/10 40	2/9 22								
<i>Ornithodoros moubata</i>	Rhesus monkey	5/5 100	5/5 100	5/5 100		5/5 100	5/5 100	7/7 100	12/12 100					
	Pig		4/4 100		4/6 66					4/6 66	2/6 33		2/3 66	
	Fowl		4/4 100		6/6 100					3/6 50		2/6 33		6/6 100

Cimex lectularius gave positive reactions up to a month after feeding; these results are difficult to compare with those obtained by Holstein (1948) who made extracts of a batch of blood meals and then tested a number of serial dilutions with precipitating antisera. The dilution of *Cimex* blood feeds corresponding approximately to 1/1000 of serum protein gave positive reactions up to 10 days. The concentration of protein of the individual test meals in our experiments was not established, but it would be unlikely that the protein concentration of the later feeds was as high as that corresponding to 1/1000 dilution of normal serum. It thus appears that the results obtained here compare very favourably with those obtained by Holstein.

Glossina morsitans (reared and fed in the laboratory) show very different results from *G. swynnertoni* collected in the field after natural feeding. Whereas almost every laboratory feed gave positive reactions after 3 days, only 7 of 25 naturally fed flies were positive when tested at the Hunger Stage III which corresponds to 2-4 days after feeding. Dr. C. H. N. Jackson (private communication) notes that, by the observations of captive flies and of marked flies in the field, it is known that blood remains microscopically recognisable for longer in captivity than in the field. Thus captive flies, although the abdomens quickly collapse through loss of water, continue to contain whole blood, which is readily seen from outside or microscopically on dissection; in the field such blood (corpuscles microscopically visible on dissection) was not visible after the second day although at this stage the abdomen would still be distended. The differences between the results obtained from laboratory-kept flies and those captured in the field correspond to these findings.

The very prolonged digestion period of ticks has already been noted by Gózonyi, Hindle and Ross (1914) and it would seem from our results that a high proportion of positive results will still be obtained some considerable time after 6 months. Among the species of blood suckers we have tested the shortest period of digestion occurs in *Bdellonyssus*.

Summary.

The period of digestion of the blood meal of 10 different blood-sucking arthropods which had been artificially and naturally fed on known hosts was studied by means of specific precipitin tests.

A large proportion of positive feed (80-100 per cent.) was found with midges and mosquitos up to 24 hours, whether naturally (*Anopheles aquasalis*) or artificially fed (*A. maculipennis atroparvus*, *Culex molestus*, *Culicoides nubeculosus*, *Aedes aegypti*).

Tsetse flies showed greater differences between the rate of digestion of captive flies (*Glossina morsitans*), which showed 96-100 per cent. positive feeds at 3 days after the experimental blood meal, and wild flies (*G. swynnertoni*) which showed only 28 per cent. positive meals after a similar period of digestion. Arachnids showed both the longest period of digestion, more than 6 months for *Ornithodoros moubata* and also the shortest period as represented by *Bdellonyssus bacoti* (about 1 day).

Cimex lectularius showed 90 per cent. positive feeds after 10 days.

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References.

- BULL C. G. & KING, W. V. (1923). The identification of the blood meal of mosquitoes by means of the precipitin test.—*Amer. J. Hyg.*, **3**, pp. 491–496.
- GÓZONY, L., HINDLE, E. & ROSS, P. H. (1914). Serological tests I.–II.—*J. Hyg.*, **14**, pp. 354–359.
- HOLSTEIN, M. (1948). Les sérums précipitants. . . —*Acta trop.*, **5**, pp. 306–326.
- WEITZ, B. (1952). The antigenicity of sera of man and animals in relation to the preparation of specific precipitating antisera.—*J. Hyg.*, **50**, pp. 275–294.
- WEST, A. S. & ELIGH, G. S. (1952). The rate of digestion of blood in mosquitoes. Precipitin test studies.—*Canad. J. Zool.*, **30**, pp. 267–272.
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ON THE EVENING BITING ACTIVITY OF THREE NEOTROPICAL *ANOPHELES* IN TRINIDAD, BRITISH WEST INDIES.

By R. A. SENIOR WHITE, F.R.S.E.

Entomologist, Malaria Division, Health Department, Trinidad, B.W.I.

Lumsden (1952) studied the evening biting activity in Central Africa in eight species of biting Diptera, one *Anopheles*, six Culicines, and a Tabanid, with regard to differences in elevation above the ground and to the time of sunset. His elaboration of 'catch time' with reference to a standard time of sunset furnished a new measurement of insect activity which greatly appealed to the present writer, who had already shown (Senior White, 1951 and 1953) that, judging by the commencement of attack, *A. aquasalis* Curry is normally activated when light incidence has fallen to an average of 4 foot-candles, and that heavy attack commences between 10 and 17 minutes *after* sunset, although the activating value is passed at any time up to 15 minutes *before* sunset, dependent upon the cloud cover of the evening sky. Attack by disturbed specimens of this species may, however, occur at any hour of the day, even in full sunlight, but this is not normal behaviour. *A. albitarsis* Lynch Arrib. appears to be activated earlier, and *A. neomaculipalpus* Curry considerably later than *A. aquasalis*, but in these species the matter has not hitherto been studied in detail. The discrepancy between the time of pre-sunset activation value in *A. aquasalis*, and the time of the real post-sunset attack is not yet understood. Table II of this paper shows that it is not a function of density fluctuations. The impression has been gained that a wet afternoon, followed by a fine evening, results in larger numbers attacking than is the case when the whole day has been fine, but there are no figures in support of this.

Lumsden commenced his catches at 15.00 hours, and continued until 21.00 hours, making 14 periods of from 10 to 60 minutes duration of which nine only were of 10 minutes each, the first of these commencing 30 minutes before sunset. In repeating his work, it was decided to use 10-minute periods throughout, and to commence catching 20 minutes before sunset, *i.e.*, 5 minutes sooner than the earliest undisturbed evening attack noted during previous work with *aquasalis*. The 27 evenings on which catches were made extended over the latest (18.25) and earliest (17.35) times of sunset at 10°N. latitude.

Lumsden used as collectors local children who caught from one another, and thus many of his captures would have taken blood before tubing. As it is not usually feasible to dissect night captures immediately, Lumsden could not perform what he styles "*the experimentum crucis*" of judging the age of attackers by their ovarian development, as this will have changed, through digestion, overnight before dissection next day. In the present experiment, captures, taken before the proboscis was inserted, were given sugar-soaked cotton to maintain them overnight. This does not lead to further development of the ovary, and it can be accepted that when dissected those captured unfed were in the same ovarian condition as at the moment of capture. If there was even a trace of blood in the gut, the specimen was tabulated only for total number captured during the period, but not for numbers in each ovarian stage.

In the present series of observations the baitmen were fully clothed (by European standards) and were wearing gum boots, from which, rather than from

(b) *Anopheles albitalarsis*.

No.	Period time	Time period worked	Ovary I					Ovary II					Ovary III & IV					Total for period	% of periods I-XII in nights' totals
			No. complete dissections	No. sperm.	F.I.	No. incomplete	Total catch	No. complete dissections	No. sperm.	F.I.	No. incomplete	Total catch	No. complete dissections	No. sperm.	F.I.	No. incomplete	Total catch		
I	17-40-17-50	27	0	0	—	0	0	13	13	100	1	14	0	0	—	0	0	14	3.4
II	17-50-18-00 Sunset	27	0	0	—	0	0	20	20	100	0	20	0	0	—	0	0	20	4.9
III	18-00-18-10	27	0	0	—	0	0	18	18	100	1	19	0	0	—	0	0	19	4.6
IV	18-10-18-20	27	0	0	—	0	0	36	36	100	2	38	0	0	—	0	0	38	9.3
V	18-20-18-30	27	4	2	50.0	0	4	51	51	100	0	51	0	0	—	1	1	54	20.6
VI	18-30-18-40	27	0	0	—	0	0	30	29	96.7	1	31	1	1	100	0	1	54	13.2
VII	18-40-18-50	27	2	1	50.0	0	2	15	15	100	0	15	2	2	100	0	2	26	6.4
VIII	18-50-19-00	27	0	0	—	0	0	8	8	100	1	9	1	1	100	0	1	12	2.9
IX	19-00-19-10	26	5	2	40.0	0	5	38	37	97.4	1	39	1	1	100	0	1	47	11.5
X	19-10-19-20	26	2	2	100	0	2	23	22	95.7	1	24	1	1	100	0	1	32	7.8
XI	19-20-19-30	26	0	0	—	0	0	27	27	100	1	28	1	1	100	0	1	32	7.8
XII	19-30-19-40	26	0	0	—	0	0	31	31	100	0	31	0	0	—	0	0	31	7.6
XIII	19-40-19-50	9	0	0	—	0	0	0	0	—	1	1	1	0	—	0	0	1	1
XIV	19-50-20-00	8	0	0	—	0	0	1	1	100	0	1	0	0	—	0	0	1	1
% of dissected sp. catch			13	7	53.8	0	13	311	308	99.0	10	321	7	7	100	1	8	411	
% of total catches			3.8					93.9					2.3					4.8	

(c) *Anopheles Neomaculipalpus*.

No.	Period time	Time period worked	Ovary I					Ovary II					Ovary III					Total for period in nights' totals	In total catch only. Not dissected
			No. complete dissections	No. sperm.	F.I.	No. incomplete	Total catch	No. complete dissections	No. sperm.	F.I.	No. incomplete	Total	No. complete dissections	No. sperm.	F.I.	No. incomplete	Total catch		
I	17-40-17-50	27	0	0	—	0	0	0	0	—	0	0	0	0	—	0	0	0	0
II	17-50-18-00	27	0	0	—	0	0	0	0	—	0	0	0	0	—	0	0	0	0
III	Sunset	27	0	0	—	0	0	0	0	—	0	0	0	0	—	0	0	0	0
IV	18-00-18-10	27	0	0	—	0	0	0	0	—	0	0	0	0	—	0	0	0	0
V	18-10-18-20	27	5	5	100	0	5	4	3	75-0	0	4	0	0	—	0	0	13	21-7
VI	18-20-18-30	27	5	5	100	0	5	6	5	83-3	0	6	0	0	—	0	0	11	18-3
VII	18-30-18-40	27	5	5	100	0	5	4	3	100	0	6	0	0	—	0	0	9	15-0
VIII	18-40-18-50	27	0	0	—	0	0	5	5	100	1	6	0	0	—	0	0	6	10-0
IX	18-50-19-00	27	3	3	100	0	3	2	2	100	0	2	0	0	—	0	0	3	5-0
X	19-00-19-10	26	1	1	100	0	1	7	7	100	0	7	0	0	—	0	0	7	11-7
XI	19-10-19-20	26	0	0	—	0	0	1	1	100	0	1	0	0	—	0	0	1	0
XII	19-20-19-30	26	3	3	100	0	3	1	1	100	1	2	1	1	100	0	1	6	10-0
XIII	19-30-19-40	26	0	0	—	0	0	4	3	75-0	0	4	1	1	100	0	1	5	8-3
XIV	19-40-19-50	9	0	0	—	0	0	1	1	100	0	1	0	0	—	0	0	1	0?
	19-50-20-00	8	0	0	—	0	0	0	0	0	0	0	0	0	—	0	0	0	0
% of dissected sp. catch			17	17	100	0	17	32	29	90-6	2	34	2	2	100	0	2	61	6
% of total catches			32-1					64-2					3-7					0-7	0-1

the clothing, the majority of the specimens were taken. To increase numbers, advantage was taken of the habit of *A. aquasalis* to rest near a blood source before actual attack and to include specimens resting on the poles supporting the thatch roof under which the party sat, and under the wooden slats of their seat. It was found later, however, that this method of collecting introduced a further variable, since if the catchers were very busy taking specimens on the baitmen the exact time of arrival of "sitters" before biting could not be noted for period. The interval between arriving to "sit" and movement for actual attack was found to be very variable, a watch on 26 specimens during a quiet night showing rests of from 10 seconds to 38 minutes prior to attack, with an average of 7 minutes 25 seconds. The reason for this rest before feeding is quite unknown; during dense periods it probably affects the numbers allocated to any particular 10-minute period.

Specimens free from blood were also dissected for fertilisation. The index so obtained is not completely accurate (Senior White, 1951), as the process of dissection may force out all the sperm from a spermatheca, and the specimen be erroneously recorded as unfertilised. In the Table, percentages are based on specimens free from blood in which the spermathecal condition appeared natural. A source of error also occurs in the allocation of some specimens to Ovary I or Ovary IIa (Senior White, *l.c.*). Any specimen showing yolk granules in the ovarioles was, in the present work, allocated to Stage II. As will be shown later, the error in allocation is about 1.6 per cent.

I found it impossible to continue work throughout the night as did Lumsden, the area used for the present experiment being isolated, and transport for the staff concerned essential. Men whose duties the next day include dawn-trap capturing at daylight cannot be kept out all night, neither can the transport driver. Except during a period of sickness, when my Inspector, Mr. G. Estwick, or my Senior Technician, Mr. G. Lewis, took charge, I was myself present on every evening, time keeping and supervising the changing of the 13 x 3 cm. coned catching tubes. The degree of concentration that the catchers have to exert is such that I am of the opinion that no longer than a two- to three-hour spell can be enforced on a single "shift." Two catchers and four actual baitmen were used, and they, together with those supervising, made up a party of 10 or 11 persons which served as the only blood attraction, as ordinarily no men or animals are in this vicinity after dark.

Studies on differences in numbers or behaviour due to elevation were not made. So far as is known, the prey of the species studied is never arboreal, though I have on occasion taken *A. aquasalis* at light in the second storey of my own residence. Lumsden was particularly concerned with attack on virus-infected monkeys sleeping in the forest canopy. In the case of a malaria-vector Anopheline, behaviour at ground level alone is usually of practical interest. Lumsden made no ground level captures.

The moving averages of numbers, used by Lumsden, and *inter alia* by the present author in other density studies, could not be used in this case as catching evenings were at weekly, or longer intervals, in view of the numerous duties of myself and my staff. Crude numbers alone are recorded, but Lumsden showed that the study of these is as significant as by transformation into logarithms.

Lumsden's work was carried out at Mongiro, Uganda, 0° 51' N., 30° 08' E., in forest, whereas the present work was done in open grass savannah with some bush and much cultivation, at Bordenal Quadrant, Trinidad, 10° 37' N., 61° 26' W. The time of sunset was taken from the Nautical Almanac for 10° N., and there is therefore a slight error in the exact time of sunset, which has not been calculated. The error is presumably constant. Watches were set the night previous by the B.B.C. time signal for 02.00 hrs. G.M.T. (= 22.00 hrs. local time). The time of the periods can be accepted as accurate to ± 1 minute. A column in Table II

TABLE II.
Attack density of *A. aquasalis* by periods I-XII.

1952	S - 20	S - 10	S	S + 10	S + 20	S + 30	S + 40	S + 50	S + 60	S + 70	S + 80	S + 90	Total catch	Moon	Lag Mins. Sunset : 1st Bite
14 Feb.	0	0	0	0	13	7	2	0	0	1	2	2	27	Nil	13
21 "	0	0	0	3	8	6	2	1	0	0	1	6	27	Nil	13
6 Mar.	0	0	0	3	13	4	2	0	2	1	2	0	27	Through	18
20 "	0	0	0	0	6	2	1	0	0	0	0	0	9	Nil	21
3 Apr.	0	0	0	0	5	3	2	0	1	0	0	0	11	Through	22
17 "	0	0	0	0	0	0	0	0	0	0	0	0	0	Nil	None
1 May	0	0	0	0	0	0	1	0	0	0	0	0	1	Through	35
15 "	0	0	0	0	2	5	1	1	0	0	0	0	9	Nil	27
28 "	0	0	0	0	1	18	1	1	1	0	0	0	22	Through	28
6 June	0	0	0	0	4	16	8	4	6	0	0	0	38	Through	24
13 "	0	0	0	0	34	172	86	24	21	22	28	37	424	Nil	21
19 "	1	0	0	2	64	67	21	30	29	17	19	27	277	Nil	-14
26 "	1	0	5	12	102	211	158	191	—	—	—	—	680	Through	-11
3 July	0	0	0	0	24	240	179	113	34	47	48	35	720	Through	?
10 "	4	2	6	36	313	404	173	22	13	55	7	55	1090	Nil	-20
17 "	0	0	2	3	40	96	104	92	47	85	32	23	524	Nil	0
24 "	0	1	1	2	93	245	150	121	35	47	15	15	725	Through	-5
31 "	0	0	3	14	136	201	165	115	52	31	32	52	803	Through	-1
7 Aug.	0	1	3	10	129	77	62	31	9	18	17	25	382	Moon I-XI	-8
21 "	0	0	0	0	70	126	166	72	54	39	18	8	553	Moon I-VII	20
28 "	0	1	0	0	20	42	30	26	6	14	12	8	159	Moon I-II	24
11 Sept.	0	0	0	0	12	45	17	20	5	6	18	3	126	Nil	21
25 "	0	0	0	0	71	44	36	7	8	5	10	7	188	Through	21
9 Oct.	0	0	0	0	28	22	15	4	3	3	4	1	80	Nil	22
23 "	1	0	0	3	123	120	32	16	16	34	20	30	395	Through	-19
13 Nov.	2	1	0	3	31	27	8	2	15	5	3	3	100	Nil	-20
20 "	2	0	0	9	53	35	11	5	2	0	1	1	119	Through	-22 ?
Total (27)	11	8	20	100	1395	2235	1433	898	359	430	289	338	7516		
% Total	0.1	0.1	0.3	1.3	18.6	29.7	19.1	12.0	4.8	5.7	3.8	4.5			
Mean	0.4	0.3	0.7	3.7	51.3	82.8	53.1	33.3	13.3	15.9	10.7	12.5	278.0		9.3

shows the duration of moonlight during the observations. Fortuitously, there were 12 evenings during which there was moonlight during the whole of the first 12 periods, and an equal number of evenings with no moon during these periods. On the remaining three evenings, the moon set during these periods. Omitting these three, there was a catch of 3,729 *A. aquasalis** made during the moonlit and 2,693 during the moonless evenings, confirming the results of the catches in the dawn traps given in a previous paper (Senior White, 1951) and showing that in this species activity is greater on moonlit than on moonless nights, even though in the present case catching only continued for 90 minutes after sunset. Lumsden, working with *A. gambiae* Giles, made no reference to moonlight, and found the only significant difference between catches to be caused by wind. As he did not measure actual velocities, his criterion for "wind" is uncertain. Going through the protocols of the present observations, there are few references to "wind," and on no occasion could an evening be described as "windy." In Trinidad, weather after sunset is nearly always calm, although it is thought that a faint air movement results in larger captures than on "dead-still" evenings.

The actual catches as set forth in Table I are divided into 14 10-minute periods, although usually only 12 periods were worked, and when numbers were high it was not feasible to dissect the entire catch. After some 30 specimens had been taken in the first pair of tubes for a period, they were replaced by a second pair, which were used, irrespective of over-crowding, to the end of the period. Only the catches in the first pair of tubes were fit for dissection the next day, and these afforded a full day's work in the laboratory. The contents of the second pair of tubes were merely counted by species, and it is due to this that none of the very few *A. oswaldoi* Peryassú tabulated was dissected as they all turned up when a second pair of tubes was in use. The local rarity of the species, or at least its low activity in the first 1½ hours after sunset, is thus shown. Only *A. aquasalis*, *A. albitarsis* and *A. neomaculipalpus* can be considered in detail. Table I can be summarised as below:—

Species	% of Total Catch	% in St. I	Fertilisation Index	% in St. II	Fertilisation Index	% in St. III	Fertilisation Index
<i>aquasalis</i> ..	94.4	18.3	53.0	79.6	98.4	2.1	100
<i>oswaldoi</i> ..	0.1	—	—	—	—	—	—
<i>albitarsis</i> ..	4.8	3.8	53.8	93.9	99.0	2.3	100
<i>neomaculipalpus</i>	0.7	32.1	100	64.2	90.6	3.7	100

The fertilisation index of *A. aquasalis* with a developing ovary in Stage II must really be 100, and so the difference of 1.6 must be caused by including in Stage II a few specimens of uncertain development, as explained previously. If these specimens are transferred to Stage I, so that the fertilisation index of Stage II becomes 100, 37 individuals are concerned. This change alters the Stage I and Stage II percentages by $1.1 \pm$, and reduces the fertilisation index of Stage I by 3.4 to 49.6 per cent. Similar changes in respect of Stage II would occur in *A. albitarsis*. In neither case would the change affect the argument. The classification of specimens with a very few yolk granules in the ovarioles must remain a question of personal opinion.

It will be noticed that the third main column in Table I (b) is entitled "Ovary III and IV." This is necessitated by the occasional capture of odd

* Actually, the difference is greater than the figures show, as on the moonlit night of 26th June the last four of the 12 periods could not be worked, owing to all the tubes taken out being full.

specimens definitely attacking in Stage IV. The need to feed again to complete an ovulation is thus not confined to *A. aquasalis*, but is found in all three species dealt with in detail in this investigation. It appears most frequently in *A. neomaculipalpus*.

Lumsden speculated as to whether the attackers of the early night might not be older, and therefore more dangerous, than those attacking later, which may be chiefly those freshly emerged, and therefore non-infectious. Accordingly, a column has been inserted in Table I (a) for *A. aquasalis* showing, for each period, the percentage of the catch found to be in Ovary I. Only an insect feeding for the first time can have an ovary in this stage, but even if a refeed is necessary to complete the first gonotrophic cycle, the ovary will, by then, have developed to Stage II. High as the adult mortality is known to be in *A. aquasalis*, it is certain that, in certain seasons at least, some of the population live to at least a second gonotrophic cycle, as proved by the presence of relict eggs in the ovary (Senior White, 1951). In the paper quoted it was shown that, with a single exception, relict eggs were found during the seasons December to February and July to September. The present series of observations ran from mid-February to late November, and therefore missed most of the cool-weather season during which the Table on p. 486 shows that relict eggs are most prevalent. In the present series relict egg findings were:—

Month	No. of times	Amount dissected	Per cent.	Periods
February	2	3 in 11	27.3	V & VI
June	5	6 in 115	5.2	VI, VIII, IX, X & XI
July	5	6 in 78	7.7	XI, XIII & XIV

The foregoing Table shows that the summer season for relict eggs is prolonged by the month of June. Proved second cycle insects may appear in any 10-minute period from sunset onwards. All were in Ovary II, none in Ovary III.

In the present state of our knowledge regarding the age of an adult mosquito, it is difficult to attempt a calculation of what percentage of an attacking population should be in Ovary I. The percentage will be affected both by intensity of breeding and by longevity. *A. aquasalis* was the only species present throughout the 27 weeks of the investigation,† and thus only this species affords material for examination of seasonal differences in the ovarian stage proportions. Tabulating only the numbers dissected, by months, we find:—

Month	No. catches	I	II	III-IV	Percentages		
					I	II	III-IV
February	2	7	56	1	10.9	87.8	1.3
March	2	14	24	2	35.0	60.0	5.0
April	2	0	10	1	0	99.1	0.9
May	3	2	28	0	6.7	93.3	0
June	4	130	360	15	25.6	71.4	3.0
July	5	256	1038	23	19.4	78.8	1.8
August	3	119	435	11	21.0	77.0	2.0
September	2	17	189	3	8.1	90.4	1.5
October	2	18	232	4	7.1	91.3	1.6
November	2	14	155	4	8.1	89.6	2.3
Totals and percentages of totals		577	2527	64	18.3	79.7	2.0

† Actually, on 17th April not a single specimen of any species attacked during eleven 10-minute periods worked that night.

Omitting consideration of the very small catches before the rains, February to May, the percentage of Stage I Ovary is high from June to August, the season of maximal breeding, indicating that many of the individuals that attack in these months are feeding for the first time: such cannot be infected or infective, and, as deduced from other considerations, the probability of malaria transmission is greater later in the year.

Thus for *A. aquasalis* between February and November Stage I is seen to average 18.3 per cent. The percentage catch by periods is well above the mean from periods II to VIII (except V) and below it from periods IX to XIV (Table I (a)). Period V is that in which the main attack commences, but with this exception the Stage I percentage does not uphold Lumsden's hypothesis regarding the attackers of the early night being on an average older, and therefore potentially more dangerous, than those that attack later than an hour past sunset. Table I (a) shows that nearly 80 per cent. of the evening attack occurs in the four periods sunset + 20' to sunset + 50'. As the table on p. 467 of my previous paper shows that 42.8 per cent. of the total night's catch occurs before 20.30 hrs. local time, which between 6th June and 5th August would fall into standard periods "sunset" to "sunset + 2½ hours" (=period XV), it can be taken that nearly 50 per cent. of the total night's attack occurs in 40 mins., at a time when the number of second or later feeders is below the mean. At this time only young children have gone to bed, and in *A. aquasalis* the majority of infections would appear to be contracted in the first two hours after sunset.

Turning now to *A. albitarsis*, Table I (b), we find a very small percentage of attackers in Ovary I. The question arises as to whether newly emerged *A. albitarsis* have a different feeding habit from that of older specimens. We have never seen the species mating (Senior White & others, 1953), and there is a big gap in the female life-history still to investigate. The periods of maximum density are sunset + 20' and sunset + 30', after which there is a sharp fall in numbers, followed by a secondary rise, which was very apparent on some individual evenings, but conditions of space preclude the presentation of twenty-seven individual protocols.

The year 1952 was a very poor one for *A. neomaculipalpus* (Table I (c)). At the commencement of these observations the species was present in some numbers, the tail-end of the prevalence of 1951, but thereafter numbers became very low. Whether this is entirely a function of rainfall is still to be studied. For what the numbers are worth, it is seen that nearly one-third of the attackers are newly emerged in Ovary I, but, unlike the two *Nyssorhynchus* species, they were all fertilised, though there must be some appreciable dissection error, as the fertilisation index of Ovary II is well below 100 per cent. As a biological problem, if not a public health one, this *Arribalzagia* merits much further study.

Summary.

Lumsden's "standard sunset" period observations have been repeated with the three commonest species of *Anopheles* occurring in Trinidad. The method was modified to yield data on the ovarian and fertilisation states of these three species over a period of ten months which included the dates of the latest and earliest sunset at 10°N.

A. aquasalis comprised nearly 95 per cent. of a total catch of 8,592 specimens. Just over 18 per cent. of those that attacked were newly emerged, and no more than half of these were fertilised. Relict eggs, denoting a second or later gonotrophic cycle, were found in February, June and July. The 40 minutes from sunset + 20 minutes yielded nearly 80 per cent. of the total of the first two hours of activity, this period comprising nearly half the estimated total night's attack. From one hour after sunset the proportion of newly emerged specimens

that attack is well below the mean. The second hour after sunset therefore provided the greatest infection risk when age, not numbers attacking, is considered.

A. albitarsis has one major activity period of 20 minutes, commencing 20 minutes after sunset. Following a well marked decrease in numbers over the next 20 minutes, attack at an increased level of intensity recommenced. Less than 4 per cent. of the total number attacking are newly emerged, and the fertilisation index of these is the same as in *A. aquasalis*. There is a gap in our knowledge of the behaviour pattern of this species from emergence to second feed.

A. neomaculipalpus showed almost one-third of the small total of captures to be newly emerged, but all were already fertilised.

References.

- LUMSDEN, W. H. R. (1952). The crepuscular biting activity of insects in the forest canopy in Bwamba, Uganda.—Bull. ent. Res., **42**, pp. 721–760.
- SENIOR WHITE, R. A. (1951). Studies on the bionomics of *Anopheles aquasalis* Curry, 1932. Part II.—Indian J. Malar, **5**, pp. 465–512.
- SENIOR WHITE, R. A., LEWIS, G. & LEE, P. (1953). On swarming and mating in *Anopheles aquasalis* Curry.—Bull. ent. Res., **44**, pp. 163–173.
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STUDIES ON BEETLES OF THE FAMILY PTINIDAE.

IX.—A LABORATORY STUDY OF THE BIOLOGY OF
PTINUS TECTUS BOIELD.

By R. W. HOWE and H. D. BURGESS.

(Plates IX and X.)

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Ptinus tectus Boield., the Australian spider beetle (fig. 1) was first found in Britain in 1892 (Chitty, 1904). It spread rapidly to become a widespread pest but there was little published information concerning its biology available prior to 1942, when this laboratory study and parallel field work (Howe, 1950a) was commenced. At that time published work was mainly German, *e.g.*, Scholz (1920), Friederichs (1932), and König (1936). The authors used specimens found in a few neglected tins of produce, such as fish food, although von Lengerken (1929) worked with large numbers found in a warehouse. All used uncontrolled temperatures, as did Braune (1948), hence their data are difficult to compare directly with the present work in which conditions of temperature and humidity have been carefully controlled. Hickin (1942a) and Ewer and Ewer (1942) showed for the first time that adult beetles need free water to drink in order to attain a good egg production. These authors, together with Gunn and Knight (1945), used controlled conditions and their results agree well with the results in this paper.

The present work comprises an attempt to evaluate the effect of food, temperature and humidity on the life-cycle, including egg production. This information is used to predict from laboratory results the areas where the species could exist and to compare them with the existing world distribution.



Fig. 1. Adult of *Plinus tectus* Boield. ($\times 14$).

Occurrence and Distribution.

P. tectus may be found in all types of storage place in a variety of products. It is commonly found in the fabric of warehouses because larvae usually pupate in cracks and small crevices and because adults tend to hide in these retreats during daylight. The insect can live and develop in accumulations of organic dust and in rodent faecal pellets. Both larvae and adults can overwinter in warehouses (cf. Mansbridge, 1936) and all stages are generally present in infestations. It has been recorded from tree sparrow nests in Germany (Kemper, 1938), from the nests of birds breeding in caves by Kemper (in Zacher, 1939b), and in Britain at the Natural History Museum, London, from pigeons' nests (Donisthorpe, 1947). Woodroffe and Southgate (unpublished) have made numerous records from the nests of pigeons and house sparrows. The pigeon nests were taken from public buildings in Basingstoke, Bristol, Guildford and in eight separate areas of London, and from dock buildings at Brentford and Portishead. The house sparrow nests were taken from houses in Bedford, Carshalton and Wallington, from public buildings in four areas of London, and from farm buildings in Buckinghamshire and Essex. They have also taken *P. tectus* from the nest of a robin in a shed at Southampton, from a starling nest on a house at Stanmore, a swallow nest on a farm in Essex, and from the nest of a swift in a London house. Woodroffe and Southgate (1951) have also recorded *P. tectus* breeding in the nests of martins and sparrows at this laboratory. Since the adult cannot fly, the abundance of the species in sheltered bird nests in all the regions investigated in England is very striking. Both pigeons and sparrows are frequent visitors to food stores so the insects could be carried by the birds themselves to nesting sites. Also the possibility that the adults reach nests by their own random nocturnal wanderings should not be underestimated.

Barrett (1942) took specimens out of doors in central Ireland in a sweepnet, and Henderson (1945) also caught a specimen in the garden of his Surrey home.

Woodroffe and Southgate (unpublished) discovered larvae feeding on dead insects in a crevice in a wooden boundary fence at this laboratory.

The range of products on which this species can feed is very wide. Hayhurst (1940) and Hinton (1941) give lists of commodities on which it has been found, including many such as wood and rubber on which it cannot breed. It spreads very quickly to all materials stored in infested premises because the adults walk rapidly and wander actively during darkness and in the dim light of sheltered places. The beetles are normally incapable of flight, most having reduced wings, but some beetles can glide fairly steeply downwards instead of falling. *P. tectus* is most usually spread over long distances by the commercial movement of infested produce and transport and possibly also by animals including man (Freeman, 1950). In fifty years it has spread widely in temperate areas and obviously has been carried about the world by ships. Nevertheless, a survey of imports into Britain (Freeman, 1948) has shown that it is rarely found on produce in ships. It is unlikely that quarantine inspectors often fail to detect a heavy infestation in cargoes although its habits can make it very difficult to find. It is more likely that a few insects gaining entry on one occasion only are sufficient to establish the species in suitable climatic areas. Most of the present infestations in Britain are endemic to the buildings and spread to most of the produce stored in those buildings.

P. tectus is widespread in temperate regions. In the following list of countries generally only one early record of occurrence is given for each country. In Europe it has been recorded from Britain, Ireland (Carpenter, 1908; Crawford, 1932), France (Lepigre, 1951), Germany (Scholz, 1920), Belgium (Mayné, 1948), Holland (Everts, 1924), Norway and Sweden (Brinck, 1945), Iceland (R. A. Davis, private correspondence), Denmark (Hansen, 1923), Finland (Hellén, 1925), Czechoslovakia (Havelka, 1945), and Russia (Shapiro, 1941). In North America, it has been found in Alaska (Hatch, 1943), Canada (Brown, 1929), especially in British Columbia (Gray, 1950), and the United States (Wilson, 1915). According to Zacher (1939a) it was found in Australia in 1846. It is regarded as a rare insect there now, but three specimens have been collected in Melbourne by F. Gay (S. W. Bailey, private correspondence). It is common in New Zealand (Belton, 1951), and was described originally by Boieldieu from Tasmania (Hinton, 1941). It has been reported from South Georgia Island in the Antarctic by Brinck (1945) and from the Falkland Islands and Tierra del Fuego by Blair (Hatch, 1933). In the U.S.A. the species has been found in as warm a climate as the coastal area of California (Linsley and Michelbacher, 1943), but there does not appear to be any other authentic record of its occurrence in areas with the Mediterranean type of climate or in hotter places, except for a single specimen collected in Kenya and in the possession of G. Beverley, Department of Agriculture, Mombasa. Records in the literature of its presence in Chile, West Africa, Natal, Turkey, Afghanistan and the West Indies have been traced to warehouse records in Britain (Richards and Herford, 1930), and Germany (Zacher, 1927), on produce imported from these places. The authors do not suggest that the produce was infested before arrival in Europe. This species is unlikely to be able to establish itself in the tropics or sub-tropics except possibly in the cooler mountainous areas.

Damage to produce.

The principal form of Ptinid damage to produce is the lowering of quality due to the presence therein of live or dead insects, silk and cocoons.

Whole grain is seldom seriously attacked. The damage to individual grains is easily recognisable, the bran covering and the endosperm immediately beneath it being eaten off unevenly (*cf.* Fraenkel & Blewett, 1943a). Ptinids are usually

restricted to the peripheral few inches of a bulk of grain and are unlikely to play any part in grain heating.

The nutritional value of food is affected only by very dense populations. An attempt was made to measure the deterioration of food due to infestation by breeding single *P. tectus* successively on the same food at 20°C. and 70 per cent. R.H. Thirty $2 \times \frac{1}{2}$ inch tubes, each containing 310 to 410 mg. of "Artox" flour, were used. In addition, successive groups of tubes each containing one insect on fresh flour were used as controls in the same desiccator. Newly hatched larvae were allocated to each tube of flour in a random manner. Little difference occurred in the length of the larval period between experimental and control insects until the eighth cycle when a retardation of about 4 days was recorded for the contaminated flour. No differences in the weight of the beetles were encountered. The small quantity of food required for the complete development of an individual *P. tectus* is shown by Gunn and Knight (1945) who bred small specimens on 2.8 mg. of flour and full sized specimens on 30 mg. The carbon dioxide output of a single beetle for the whole of its development is equivalent to the oxidation of 4 to 5 mg. of carbohydrate (Howe, unpublished). An average adult weighs about 3.0 mg.

Containers made of sacking, cardboard or paper may be holed by larvae chewing through them, especially when preparing sites for building cocoons (*cf.* Spoon & Loosjes, 1947). Damage to containers by adult beetles is unusual. Wood, either of boxes or barrels or as part of the structure of buildings, is often damaged by mature larvae enlarging cracks or depressions as sites for cocoons. Musgrave (1946) recorded extensive damage to leather by larvae tunnelling into it. Hickin (1942b) records penetration of cellophane by wandering larvae and by starved adults. Ordinary jute sacks are easily penetrated, but linen sacks are more resistant.

Some laboratory experiments were performed with a few small coarse linen bags to investigate the movement of various stages of *P. tectus* through bags. Each bag was filled with flour, weighing about 1.5 kg. when full. Twenty adult beetles were placed inside one bag and only one was able to escape to the outside, while none of the twenty beetles placed outside another bag penetrated it, although a few offspring were found inside from eggs laid through the linen. Fifty eggs were placed inside each of another four bags and allowed to develop. In three of these bags all the larvae pupated inside the bags. Cocoons were mostly spun against the linen, the seams being the usual site. In the fourth bag 34 larvae pupated on the outside. This bag was not opened until all the adults died and none of the adults from larvae that pupated inside the bag escaped from it. In experiments with eggs placed on the outside of bags most of the larvae penetrated the bags and pupated inside, only a few pupating on the outside.

In another experiment, 120 young larvae were scattered over the surface of 420 cubic inches of wheatfeed, 5 ins. deep in a battery jar. During their development they penetrated throughout the food. Generally, *P. tectus* larvae remain in the outer layers of sacked produce, but they are able to penetrate to the middle of sacks (Howe, 1950a).

Natural enemies.

Two parasites of *P. tectus* have been recorded in the literature, *Dimachus discolor* (Wlk.) by Zacher (1933) and *Cephalonomia quadridentata* Duch. which was found to accept *P. tectus* experimentally by van Emden (1931). Hickin (1941) recorded the predator, *Hypoaspis* sp., and the non-predatory mite, *Thyreophagus entomophagus* (Lab.), in cultures. In the present work *Cheyletus eruditus* (Schr.) has occasionally been found in cultures. There is no reason

to suppose that any predatory species would avoid *P. tectus* but in Britain the species is comparatively free of parasites (Howe, 1950a).

The non-predatory mite, *Glycyphagus destructor* (Schr.), has occasionally affected experimental cultures of *P. tectus* and presumably caused the heavy mortality of larvae and pupae usually noted on these occasions.

Statistical Methods.

Experimental data have been summarised and tabulated as the mean (M) and standard error of the mean (S.E.) for the number (N) of insects examined using the methods given in Chapter V of Fisher's text-book (1946). When experiments included two samples, the means were compared by a "t" test and the form $t = (M_1 - M_2) / \sqrt{(S.E._1^2 + S.E._2^2)}$ was used together with the table of "t" from Fisher (1946, p. 174). This test measures the probability (P) that a difference between means equal to or larger than that observed could have occurred by chance in samples taken at random from two populations having the same mean.

Many experiments involved more than two groups simultaneously. Means of groups were then compared by the methods of analysis of variance described by Mather (1949), Chapter VI, and the range test for statistical significance (David, 1951). Regression lines were calculated and their significance determined by the methods given by Mather (1949). Tests for differences in variance, a measure of the variability of data are given in Fisher, Chapter VII.

Throughout this paper differences between means or variances are said to be significant (meaning statistically significant) if the value of P is below 0.05, i.e., that such a difference would occur by chance in 5 per cent. or less of experiments, if in fact no real difference existed.

Since examinations of insects were made only once daily, significance tests used for short periods are not strictly valid because the results are experimentally collected into very coarse groups.

Life-cycle.

The stages of the life-cycle of *P. tectus* (fig. 1 and Pl. IX, figs. 1-4) are very similar to those described for other Ptinids by Howe and Burges (1952). At 70 per cent. R.H. the percentages of the developmental period occupied by the egg, larval, pupal and pre-emergence stages at 20° and 25°C. are, respectively, 12, 56, 17 and 15, and 13, 56, 16 and 15.

The larva is included in a key to the larvae of Ptinids by Manton (1945); it usually has three moults, the first and second instars being of about the same duration and shorter than the third instar. In favourable conditions not more than seven per cent. of the larvae have an extra moult and the duration of the extra instar is very variable. The proportion of larvae experiencing extra moults is increased by temperatures above the optimum (e.g., 29.5°C., see p. 474) and by low humidities. These factors may induce more than one extra moult. At 40 per cent. R.H. and 25°C. only three out of 34 newly-hatched larvae completed the second instar. Of these three, one had two extra moults before pupating and two had three extra moults, one larva pupating and the other dying. Both pupae died. Howe and Burges (1952) found that poor food may also cause extra moults in other Ptinid species.

Like other Ptinid species, the feeding *P. tectus* larva extrudes a mucous thread containing faecal particles which often forms an incomplete envelope around the larva. Feeding is completed about three-quarters of the way through the third instar when, after finding a site for pupation, the larva builds a strong

cocoon with silk passed from the anus. According to Vajropala (unpublished)† the silk is secreted by the Malpighian tubes and mesenteron. The cocoon is tough but not brittle and incorporates little food. When it is spun against the glass of a specimen tube the occupant is clearly visible. If cracks are available, the fully-fed larvae will spin their cocoons in them. An attempt was made to find the size of crack preferred by using wooden slabs separated by layers of perspex. The larvae showed no definite preference, but enlarged small cracks by chewing away the wood and in wide cracks built cocoons in the angles between the wood and perspex. The feeding larva is negatively phototropic but the fully-fed larva in search of a pupation site does not avoid the light.

Diapause has not been recorded in *P. tectus*.

In Tables VI, VIII, X and XI, which give the length of the developmental stages, the mean of the total developmental period frequently does not exactly equal the total of the means of the constituent periods. This is due to two causes. Firstly, the figures given for immature stages are based on all those insects completing the instar although some may have died before becoming adult and so are not usable in calculating total development. Secondly, it is usual for a few larvae to have extra instars.

For all sets of conditions, the mean weights of female beetles are consistently higher than those of males, the difference frequently being statistically significant. Nevertheless, there is often a considerable overlap and the larger males are always heavier than the smaller females. No difference between sexes has been noticed for the duration of any stage of development.

Development.

Experimental methods and apparatus.

The method of culturing the beetles used to provide eggs for developmental experiments is given by Howe (1949a). The foods usually used for cultures were wheatfeed, flour and fishmeal, sometimes with the addition of 5 per cent. by weight of yeast. A mixture of 10 per cent. fishmeal with wheatfeed was also used. Cultures were kept at a relative humidity between 60 and 80 per cent. and at uncontrolled room temperatures (about 15° to 20°C.). Before beetles were used for egg laying, they were always allowed access to free drinking water for several days.

The chief experimental temperatures were 35°±0.5°C., 30°±1°C., 28.2°±1°C. and 26.8°±0.5°C. maintained in incubators; 33°±0.5°C., 25°±0.5°C., 23°±0.5°C., and 19.7°±0.5°C. (hereinafter referred to as 20°C.) in constant temperature rooms; 15°±0.5°C. and 13°±0.5°C. in incubators placed inside a refrigerator and temperatures between 10° and 2°C., to within 1°C. in refrigerators. Still lower temperatures were obtained in the ice box of a refrigerator, the actual temperatures and ranges being determined by means of thermocouples. The humidity of the constant temperature rooms was controlled at 70 per cent. R.H., making it possible to perform experiments at 70 per cent. R.H. at a number of temperatures in jam jars, 2×1 inch glass specimen tubes, and in various other ways on a fairly large scale. For all experiments using 2×½ inch glass tubes, combinations of temperature and humidity were obtained in both incubators and constant temperature rooms by the use of desiccators, the humidity being controlled by solutions of caustic potash of appropriate density (see Solomon, 1951). Distilled water was used to obtain approximately 100 per cent. R.H. and a saturated solution of zinc chloride for 11 per cent. R.H. There is some doubt as to the precise humidity given by this last

† Vajropala J. (1934). A histological study of the production of silk by the larva of *Ptinus tectus* Boield., Coleoptera, Ptinidae. Ph. D. thesis filed in Huxley library, Imperial College of Science and Technology, No. 97.

solution, but it is close to 11 per cent. Desiccators were aired regularly and caustic potash solutions renewed about every six weeks.

Eggs were incubated in 2×1 inch tubes without food and newly-hatched larvae were placed on food with a soft paint brush. The top of each tube was covered by cotton cambric held in place by a cork with a hole in the centre. Food in excess of the amount required for complete development was provided from the beginning, except in experiments involving examinations for larval moults. In these, only a small amount of food was provided for the newly-hatched larva. Then the quantity was regularly increased according to the size of the larva, usually after each moult. For all experiments food was stored before use in thin layers at the experimental conditions of temperature and humidity for at least one week for moisture conditioning.

The tubes were examined daily at about the same time. Experience allowed examinations to be omitted when no change was likely to occur. Assuming no diurnal rhythm this allows a maximum error of one day in every period. As the numbers of insects used increases, this error approaches zero for the mean period.

Disturbance of the tubes during examination was minimised, especially while the larvae were spinning cocoons, as disturbance interferes with this process.

The adult beetles were weighed on the day of emergence from their cocoons using a balance accurate to 0.1 mg. and then sexed by gentle dorso-ventral pressure which causes the aedeagus or ovipositor to protrude.

Variability of the insects.

1. General variability.

The mean values for larval and total developmental periods and weight of adult beetles in a series of eight consecutive experiments with a standard food, at approximately 20°C. and 70 per cent. R.H. are given in fig. 2. These results show that even in experiments repeated under conditions kept identical as far as possible, values for the developmental period and adult weight may differ significantly from experiment to experiment.

Twenty larvae were used in each experiment. Each larva was bred alone in a $2 \times \frac{1}{2}$ inch tube on the same measured volume of fresh "Artox" wholemeal flour provided by Dr. D. L. Gunn (see Gunn & Knight, 1945). As soon as each experiment was finished the next was started. Thus experiments were carried out with groups of newly-hatched larvae from different sources. The series of experiments occupied a period of two years and the same stock jar of flour was used throughout.

The mean of the temperature actually experienced during each experiment is plotted in fig. 2. Over the first seven experiments the mean temperature of the room during each experiment varied between 19.3° and 19.8°C., but when the eighth experiment was in progress a prolonged heat wave forced the mean temperature up to 20.3°C. This temperature rise was accompanied by some acceleration of developmental speed in this particular experiment, but over the whole series the slope of the regression line of the speed of larval development on temperature was not significantly different from zero.

The results of this series of experiments were treated by the analysis of variance technique. For all the attributes considered, namely, the developmental period of larvae and pupae, the period spent as an adult in the cocoon, and the weights of the adults produced, the variance between the experiments was found to be significantly greater than the variance within the experiments.

This variability between experiments must be the sum total of all the varying factors inherent in experiments carried out at different times. These factors include the variations in mean temperatures from one experiment to the next, which although it is the factor most likely to affect the developing larvae, is shown above not to be an overriding systematic factor. For each experiment new solutions were made to control relative humidity which may vary slightly from experiment to experiment. This is unlikely to have any great effect because the changes caused by altering humidity in the region of 70 per cent. R.H. are not large (see fig. 7).

The groups of insects used may vary considerably genetically and in general health. In the first six experiments the same insect stock was used, but in these the various means differ between themselves as much as from the other two experiments for which two different stocks were used. It is not likely that any marked change from generation to generation occurred in these culture stocks. Gunn and Knight (1945) obtained no significant difference in development period between two different stocks of insects. Howe (1950b) obtained some evidence that the age of the parent when the egg is laid has an effect on the development period. No record of the age of the parent was made in this experiment but probably the parental ages were very mixed. The season of egg laying had no discernible effect.

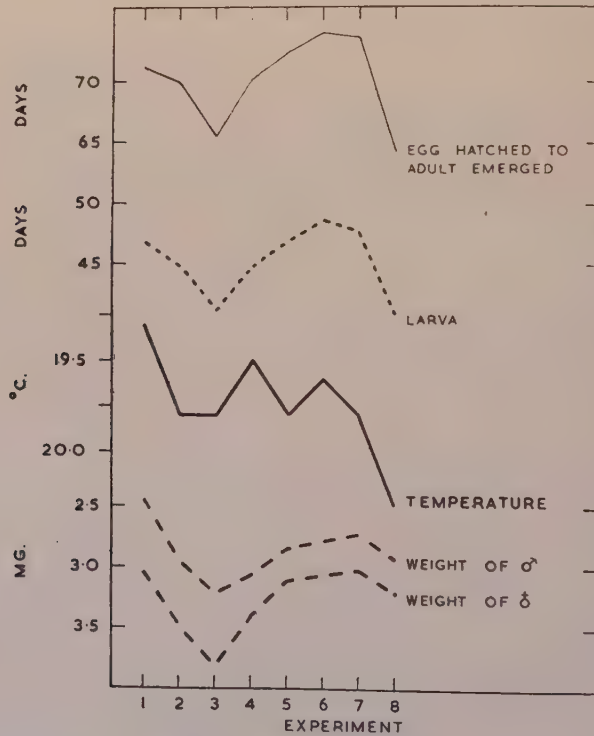


Fig. 2.—Variation of results.

TABLE I.

Developmental period (hatching of egg to emergence of adult from cocoon) at 70 per cent. R.H.

	Gunn & Knight (1945)		Howe & Burges	
	24.7°C. M	25.0 N ϕ	\pm 0.5°C. M ϕ \pm S.E. ϕ	
Fishmeal	58.5	20	60.3 \pm 0.9	
Wholemeal flour	56.4	21	64.3 \pm 5.8	
Wholemeal flour + 5% yeast ..	51.2 to 54.5 (10 expts)	23	51.7 \pm 0.6	

Comparison of results of present work with those of Gunn & Knight (1945).

ϕ In this and in succeeding Tables N=number of insects on which the mean (M) is based; S.E.=standard error.

There is no systematic change with time in the experimental results (fig. 2) so that no deterioration of the flour in storage is likely to have occurred.

Thus, although there was enough variability between different experiments in this series to give significant differences between some pairs of means, this variation cannot be ascribed to any single major cause.

Gunn and Knight (1945) found that the mean development period in ten experiments ranged from 51.2 to 54.5 days, but did not indicate the internal variation within each experiment, so the significance of this range cannot be judged. Table I compares results from the present work and that of Gunn and Knight (1945) under similar conditions showing both similar and dissimilar results according to the food used.

In experiments generally, every cause of variation was removed as far as possible. Since there is this tendency for differences to occur in experiments carried out at different times the effect of temperature, humidity, and food were investigated by means of experiments carried out *simultaneously* and most experiments were accompanied by controls at 25° or 23°C. and 70 per cent. R.H. with fishmeal or wheatfeed as food. In each experiment eggs for all conditions used came from a single source. Food was purchased in large quantities which lasted for experiment and culturing for long periods. When a new lot of food was introduced the old and new were compared using thirty larvae on each food. Thus, when a new lot of fishmeal was introduced, the larval development period on the new lot was found to be 41.4 days at 25°C. and 70 per cent. R.H. compared with 40.3 days on the old lot, a difference not significant statistically. Before use, all foods were heat sterilised at 60°C. for 4 hours in closed kilner jars. This process did not affect the food value, as shown by the length of the developmental period of the larva on sterilised and unsterilised samples.

2. Variability of closely related beetles.

A large number of offspring from single pairs of adult beetles were bred in order to investigate the factor of parentage in insects developing simultaneously in standard conditions.

In one experiment, inbreeding was carried out for two generations at both 23° and 25°C., then for two additional generations at 25°C., using pairs of beetles from both temperatures. When selecting pairs, as far as possible beetles of similar weight or with similar developmental periods were paired.

The importance of carrying out comparative experiments simultaneously is emphasised by the large differences in developmental period which occurred between the different generations, possibly due to environmental variations such as temperature and food, and to handling. Because of these differences separate analyses of variance were carried out for each generation at each temperature.

The values obtained and the results of these statistical analyses are given in Table II.

The mean larval development periods of the offspring of different pairs in a generation varied widely as did the weights of the adult offspring. These differences were much greater than would have been obtained by the random selection of insects from a homogeneous population, most of the differences being significant at the 1 per cent. level. Even when comparisons were made between offspring of related pairs in a generation, *i.e.*, descended from one of the original first generation pairs, the differences were statistically significant in 8 out of 13 instances for the larval period, in 4 out of 13 for the weight of female beetles and in 9 out of 12 for the weight of males.

No significant relationship between the mean larval developmental period of the offspring and that of the parents was discovered in any generation. On the other hand, the slope (*b*) of the regression line relating the mean weights of offspring and of parents was significantly different from zero at the 1 per cent. level in seven out of eight instances, indicating a real relationship between the two.

TABLE II.

Larval period in days and weight in milligrams of offspring of pairs of *P. tectus* at 70 per cent. R.H. on fishmeal.

Temp. °C.	Generation	Parents		Offspring			
		Ancestral pair	No. of parent pairs	Larval period N. M.	Weight of females N. M.	Weight of males N. M.	
23	I		3	104 60++	39 3.83+	59	3.41
23	II	A	7	297 65++	146 3.75	134	3.44++
"	"	B	2	82 64++	39 3.96+	40	3.57++
"	"	C	3	323 59++	153 3.42++	161	3.15++
			12	702 62++	338 3.65++	335	3.32++
25	I		4	151 53++	70 3.68	70	3.40++
25	II	D	10	253 64++	113 3.47	130	3.18++
"	"	E	3	99 65	43 3.35	51	3.16
"	"	F	3	50 70++	22 3.57	20	3.19++
"	"	G	1	43 67—	20 3.85—	26	3.35—
			17	445 65++	198 3.49++	227	3.20++
25	III	A	4	81 55++	27 3.36+	37	3.08
"	"	B	2	26 53	7 3.24	7	2.67—
"	"	C	3	37 52	13 2.52	7	2.31++
"	"	D	2	13 57+	7 3.13	4	2.38++
"	"	E	3	49 48	16 3.06	15	2.65+
"	"	G	1	10 50—	4 3.00—	7	2.79—
			15	216 53++	74 3.09++	77	2.83++
25	IV	A	2	39 57	16 3.14+	24	2.99
"	"	E	3	42 51++	21 3.25	19	2.71+
			5	81 54++	37 3.20	43	2.87+

Statistically significant differences between offspring of the pairs in each generation are indicated by two crosses for the one per cent. level and one cross for the five per cent. level of significance. A dash indicates that no comparison is possible.

Temp. °C.	Generation	Females		Males	
		b	P	b	P
23°	II	0.847	<0.01	0.721	<0.01
25°	II	0.855	<0.01	0.598	<0.01
25°	III	0.244	<0.01	0.279	<0.01
25°	IV	0.185	0.1	0.392	<0.01

In genetic terms this would suggest that genes controlling weight may segregate more quickly than those controlling developmental period.

There was no indication that the variation of the offspring of a pair of beetles was decreased by inbreeding for four generations.

The differences in the developmental period and weight of offspring of a number of unrelated pairs of beetles in concurrent experiments at 23°C. and 70 per cent. R.H. on fishmeal are shown in Table III. These also differ widely.

At 13°C. and 70 per cent. R.H. the egg incubation periods of two batches of eggs from two different groups of adults differed significantly; 22.5 days as compared with 28.0 days. Such a difference is presumably genetic, since it cannot be attributed to temperature or any other known cause.

TABLE III.

The developmental period and weight of adult beetles bred from eggs laid by separate, unrelated pairs of adults.

Parental pair of beetles	Larval developmental period of offspring in days			Weights of adult offspring in mg.					
				♀♀			♂♂		
	N	M	S.E.	N	M	S.E.	N	M	S.E.
1	45	47.5	±0.6	19	3.42	±0.05	18	3.09	±0.06
2	56	48.0	±0.9	29	3.46	±0.08	23	3.12	±0.07
3	58	48.0	±1.3	21	3.41	±0.10	28	3.25	±0.08
4	45	48.2	±0.6	17	2.70	±0.10	12	2.61	±0.09
5	55	48.2	±0.7	20	3.58	±0.10	21	3.23	±0.05
6	56	48.8	±0.7	26	3.14	±0.09	26	2.88	±0.05
7	77	49.6	±0.7	30	3.14	±0.06	32	2.99	±0.08
8	38	50.7	±0.7	14	3.19	±0.10	13	2.88	±0.12
9	73	51.0	±0.6	26	3.22	±0.06	25	2.96	±0.06
10	41	53.0	±0.8	14	3.26	±0.10	20	3.08	±0.06
11	59	53.5	±0.8	20	3.54	±0.09	22	3.22	±0.06
12	69	55.0	±0.7	31	3.15	±0.07	24	2.89	±0.05
13	28	55.6	±1.7	9	2.97	±0.09	11	2.81	±0.08
14	51	55.7	±1.4	24	3.11	±0.06	21	2.73	±0.05
15	64	58.5	±1.1	23	3.36	±0.09	27	3.00	±0.09

The insects were bred on fishmeal at 23°C. and 70 per cent. R.H.

Temperature.

Complete development of *P. tectus* is possible within a temperature range extending from just over 5°C. to 28°C. The most rapid development occurs between 23° and 27°C.

At temperatures above 38°C . death ensues in a few hours (fig. 3). The effect of these high temperatures was investigated using groups of five adults or of five third instar larvae exposed in small muslin bags of about 1 cc. capacity. At the beginning of exposure the temperature experienced by the insects rose from room temperature ($15^{\circ}\text{--}20^{\circ}\text{C}$.) to the experimental temperature in seven minutes, then

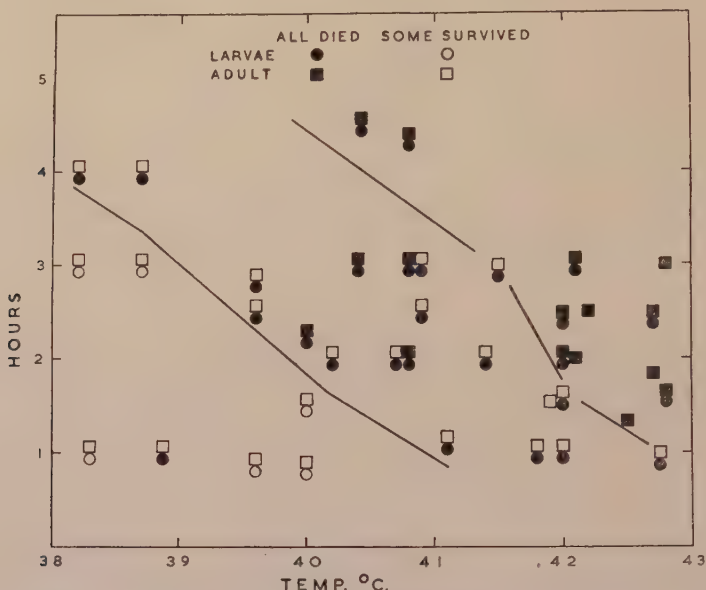


Fig. 3.—The resistance of adults and large larvae of *P. tectus* to rapidly lethal high temperatures. Each symbol represents an experiment with five individuals.

fluctuated by 0.3°C . or less about the mean temperature. After exposure the treated insects were placed at 20°C . Adults dying in less than 14 days and larvae failing to reach the adult stage were considered to have been killed by exposure to the high temperature. No deaths were recorded among the insects kept as controls. The larvae were slightly less resistant than adult beetles to these high temperatures. Adults were killed by two hours at 42°C . and by four hours at 41°C . Larvae were killed by one hour at 41°C ., and by four hours at 38°C .

Larvae, pupae and adults were exposed to 35° and 33°C . using the same methods as for life-history experiments at lower temperatures, and were left until they died. Third instar larvae were less resistant than pupae to both temperatures, but it is possible that the periods given for pupae may be too large as it is very difficult to decide when pupae are dead. Adults were less resistant to 35°C . but more resistant to 33°C . than larvae or pupae. The mean lengths of survival in days of the three stages (taken from Tables XII, XIII and XXV) were:—

	Larvae	Pupae	Adults
35°C .	7.8	10.0	5.9
33°C .	11.5	12.1	13.5

Whereas Ewer and Ewer (1942) obtained no hatching at any humidity at 29.5°C., in this work 31 per cent. of eggs hatched at 30°C., and 70 per cent. R.H. (Table IV.)

TABLE IV.

Reaction of eggs to exposure for various periods to unfavourable combinations of temperature and humidity within 24 hours of laying.

Temp. °C.	R.H. %	Exposure in days	Number of eggs exposed	% eggs hatched on return to 25°C. & 70% R.H. (or at exp. condition \emptyset)	Incubation period days M \pm S.E.
35	70	3	10	0	
33	70	3	10	10	
33	70	5	5	0	
30	20	3	10	70	
30	20	5	10	30	
30	20	7	5	0	
30	20	9	5	0	
10	20	9	10	80	
10	20	17	10	80	
10	20	28	10	90	
10	20	41	10	10	
10	20	53	10	0	
3 \pm 1	70	10	74	40	
3 \pm 1	70	38	21	0	
3 \pm 1	70	53	19	19	
2 \pm 2	not controlled	29	294	35	
30	70	{ until hatching or death	81	31 \emptyset	8.7 \pm 0.2
25	70		20	90 \emptyset	9.3 \pm 0.5
20	20		46	59 \emptyset	17.6 \pm 0.4
10	70		41	22 \emptyset	44.0 \pm 0.9

Larvae kept continuously at 30°C. all died at both 100 per cent. and 70 per cent. R.H. Exposure of older larvae to 30°C. for 6 days or less had no effect on the duration of larval development but exposure for 8 days or more retarded the speed of larval development. Exposure late in larval life increased the pupal period and reduced the adult weight (Table V), but had no effect on the pre-emergence period.

TABLE V.

The effect of exposing groups of 10 larvae of different ages (reared at 20°C.) to 30°C. and 70 per cent. R.H. for various periods, then returning them to 20°C. Wheatfeed was present throughout.

Group	Exposure at 30°C.	Age at 20°C.	Mortality (% died)	
			Larval	Pupal
A	whole life	0 days	100	—
B	2 days	2 "	0	0
C	4 "	2 "	0	0
D	8 "	2 "	70	0
E	12 "	2 "	60	0
F	6 "	15 "	10	0
G	12 "	15 "	0	10
H	18 "	15 "	30	0
J	12 "	20 "	30	30
K	20 "	20 "	0	10
L	0 "	control	0	0

	Groups	Total period in days at 20° and 30° C. N M ±S.E.		Comparison with control P.
Larval Period				
6 days or fewer at 30° C.	B, C, F	29	41.8±2.3	>0.9 0.01
8 days or more at 30° C.	D, E, G, H, J, K,	41	54.1±2.0	
Control at 20° C.	L	10	41.6±4.0	
Pupal Period				
Larva 2 days at 20° C. before exposure ..	B, C, D, E	26	12.5±0.1	0.7
Larva 15 days at 20° C. before exposure ..	F, G, H	24	12.7±0.1	0.7
Larva 20 days at 20° C. before exposure ..	J, K	11	13.5±0.2	0.01
Control at 20° C. ..	L	10	12.7±0.2	

Adult		Weight in mg.		Comparison with control P
		♀♀	♂♂	
Short exposures, younger larvae	B, C, D, E, F, G	25 3.18 ± 0.06	19 2.90 ± 0.05	0.2
Longer exposures, older larvae	H, J, K	9 2.59 ± 0.10	11 2.41 ± 0.06	0.001
Control at 20° C.	L	5 3.38 ± 0.13	4 3.07 ± 0.11	

Larvae transferred to 29.5°C. and 70 per cent. R.H. after 10 days at 23°C. and 70 per cent. R.H. all died before becoming adult, only one reaching the pupal stage. Many of these larvae had reached the second instar before transfer and all the rest survived the first moult. The details of developmental period in days and mortality including the period at 23°C. are:—

	N	M	±	SE	% Mortality
Larva I	29	10.8	±	0.2	0
Larva II	19	14.0	±	1.1	32
Extra instar	4	30.0	±	8.5	} 64
Larva III	1	42			
Last moult (before dying) to death ..	27	33.2	±	4.5	
Pupa	—	—	—	—	4

Four out of five larvae reaching a sufficient age had more than the three moults usual at lower temperatures.

At 29°C., and 70 per cent. R.H. newly-hatched larvae transferred from 23°C. and 70 per cent. R.H. died in the first or second instar. Two larvae moulted in 10.5 ± 1.5 days. The period to death was:—

	N	M	±	SE	% Mortality
Hatching to death, larva I	28	15.6	±	1.0	93
Moulting to death, larva II	2	7.5	±	2.5	7

Thus it is quite clear that young larvae cannot survive if the temperature does not fall below 29°C.

At 28.2°C. (Table VI) development was completed but mortality was high (78.2 per cent.). The developmental period was longer than at 26.8°C. and the

TABLE VI.
Developmental period on wheatfeed at 70 per cent. R.H.

Stage	20° ± 0.5° C.			26.8° ± 0.5° C.			28.2° ± 1.0° C.		
	N	M ± S.E.	% died	N	M ± S.E.	% died	N	M ± S.E.	% died
Egg ..	25	10.8 ± 0.5	50.0	82	7.1 ± 0.1	50.0	64	7.7 ± 0.6	48.4
Larva I ..	9	9.1 ± 0.2	—	24	6.4 ± 0.02	2.5	16	8.4 ± 0.4	10.4
Larva II ..	9	10.4 ± 0.2	—	20	7.7 ± 0.3	—	14	8.6 ± 0.4	—
Extra I ..	—	—	—	—	—	—	5	12.4 ± 1.2	—
Extra II ..	—	—	—	—	—	—	1	12	—
Larva III ..	8	26.0 ± 0.8	—	21	23.1 ± 1.3	2.5	10	27.5 ± 1.6	12.9
Total larva *	19	44.1 ± 0.5	6	62	38.5 ± 1.0	7.5	24	48.2 ± 1.7	23.4
Pupa ..	14	13.4 ± 0.1	—	56	8.9 ± 0.1	3.1	19	9.4 ± 0.9	3.2
Pre-emergence ..	14	10.4 ± 0.4	2	52	7.6 ± 0.3	1.2	16	8.7 ± 0.9	3.2
Egg laid to adult emerged ..	18	77.9 ± 0.7	58	61	61.0 ± 0.9	61.8	19	69.5 ± 1.8	78.2
Weight of ♀♀ (mg.) ..	9	3.03 ± 0.05	—	38	2.40 ± 0.03	—	9	1.91 ± 0.10	—
Weight of ♂♂ (mg.) ..	9	2.66 ± 0.07	—	17	2.23 ± 0.09	—	10	1.80 ± 0.03	—

* Comprises two sets of larvae, one undisturbed and the other examined for larval moults.
A significant difference occurred between the two sets only at 20°C. (43.0 and 45.6 respectively;
P = 0.02).

beetles were very light in weight. At 26.8°C. post-embryonic mortality was less severe than at 28.2°C. and the developmental period was about the same as at 25°C. In another experiment, however, the developmental period for the larva at 27°C. was found to be similar to that at 20°C. This is in closer agreement with the results of Ewer and Ewer (1942). Table VII shows the results of this experiment in which larvae of various ages were transferred from 20°C. to 27°C. The pupal and pre-emergence periods were shorter at the higher temperature. Transfer of larvae to 27°C. was apparently followed by a short spell of accelerated development after which the rate of development fell back to that at 20°C. Thus, for instance, larvae transferred at the end of the first and at the beginning of the second instar completed the second instar in 7.7 and 7.5 days respectively as compared with 8.4 days or more in the other groups.

TABLE VII.

Developmental period in days on wheatfeed at 20° and 27°C. and 70 per cent. R.H.

Period at 20° C. before transfer	Larval instar at transfer to 27° C.	Total period as larva at 20° and 27° C.		Weight of adults produced (mg.)			
		N	M±S.E.	Females		Males	
		N	M±S.E.	N	M±S.E.	N	M±S.E.
5	Middle I	60	41.0±2.7	2	1.91±0.35	4	1.98±0.19
7	Late I	4	38.2±0.6	—	—	4	1.90±0.09
11	Early II	10	43.1±2.0	4	2.30±0.09	2	1.80±0.10
19	Early III	10	41.7±1.2	4	2.40±0.34	5	2.38±0.16
31	Middle III	8	40.2±1.9	3	2.73±0.09	5	2.30±0.15
Whole life	—	10	40.8±3.4	6	2.82±0.13	4	2.98±0.11

	27° C.			20° C.			
	N	M	S.E.	N	M	S.E.	P
Pupa	32	8.7 ± 0.1		10	12.7 ± 0.1		0.01
Pre-emergence	32	9.0 ± 0.7		10	10.5 ± 0.3		0.01

φ For the first four days at 27°C. the temperature rose each day to 29°C. and fell back to 27°C. each night.

At 25°C. and 23°C. the total developmental period from egg laid to adult emergence is usually about 65 to 70 days on fishmeal (Table VIII) and five to ten days less on wheatfeed. Development takes progressively longer as the temperature falls below 23°C. (Tables VIII, IX, X, XII and XIV).

The lowest experimental temperature at which development was completed was 11°C. (Table VIII). Nevertheless eggs hatched at 10°C. (Table IV) and at 5°C. very slow larval and pupal development was recorded in an experiment in which larvae more than half grown at 25°C. were transferred to this temperature (Tables XII and XIV). Half of these larvae (20) died before becoming adult and all pupae (7) moved to 5°C. from 25°C. after pupation (Table XIII) died without becoming adult. At 3°±1°C., eggs did not hatch in 53 days, but some were still alive because a few hatched after removal to 25°C. In another experiment newly-hatched, late first, early second and middle third instar larvae (15 of each age) bred at 20°C. were exposed to 3°C. for 30 days and then returned to 20°C. The results are not tabulated because only among newly-hatched larvae was the mortality high, nine dying. In all groups development appeared to be completely arrested. Thus the minimum temperature at which complete development can occur is put tentatively between 5° and 10°C.

TABLE VIII.

The developmental periods in days at several temperatures on fishmeal at 70 per cent. R.H.

Temp. °C.	Egg		Larva		Pupa		Pre-emergence		Total, egg laid to adult emerged from cocoon	
Mean Range	N	M \pm S.E.	N	M \pm S.E.	N	M \pm S.E.	N	M \pm S.E.	N	M \pm S.E.
11 \pm 2	24	24.5 \pm 0.19	10	237.5 \pm 8.20	2	34.0 \pm 0.00	1	12.0 —	1	330 —
15 \pm 0.5	68	17.5 \pm 0.08	64	78.0 \pm 0.44	63	21.8 \pm 0.16	59	13.0 \pm 0.27	60	130.2 \pm 0.16
21 \pm 1.0	108	8.6 \pm 0.07	99	59.9 \pm 0.60	93	15.9 \pm 0.08	84	9.5 \pm 0.14	84	93.7 \pm 0.65
23 \pm 0.5	76	9.4 \pm 0.11	43	49.7 \pm 0.93	27	11.4 \pm 0.15	19	8.3 \pm 0.63	20	78.3 \pm 1.14
23 \pm 0.5	—	— —	15	37.5 \pm 0.93	12	10.9 \pm 0.55	11	9.2 \pm 0.92	11	67.0 —
25 \pm 0.5 mean of 8 expts.	155	7.9	211	44.4	175	9.8	76	6.4	76	67.2
max. —	72	8.0 \pm 0.13	71	45.3 \pm 0.76	4	10.2 \pm 0.25	4	8.7 \pm 0.63	51	69.4
min. —	83	7.8 \pm 0.12	4	40.0 \pm 0.91	5	8.8 \pm 0.58	12	5.7 \pm 0.48	27	62.3

TABLE IX.

The length of the larval instars in days at several temperatures on fishmeal at 70 per cent. R.H.

Temp. °C. Mean	I		II		Extra		III	
Range	N	M ± S.E.	N	M ± S.E.	N	M ± S.E.	N	M ± S.E.
11 ± 2	14	35.7 ± 0.38	13	35.5 ± 1.33	1	12 —	10	164.9 ± 7.56
15 ± 0.5	68	15.5 ± 0.11	66	18.4 ± 0.10	—	— —	64	44.1 ± 0.34
21 ± 1.0	99	12.1 ± 0.25	99	16.2 ± 0.20	5	16.4 ± 1.74	99	30.7 ± 0.32
23 ± 0.5	47	16.6 ± 0.32	44	11.0 ± 0.14	5	10.5 ± 0.40	44	25.9 ± 0.47
23 ± 0.5	14	7.4 ± 0.27	14	8.9 ± 0.16	1	7.0 —	15	21.1 ± 0.87
25 ± 0.5	331		317	9.7	11	10.8	212	26.0
Mean of 8 expts.	61	9.2 ± 0.13	57	11.4 ± 0.14	3	14.3 ± 0.41	71	28.5 ± 0.75
max. —	56	7.3 ± 0.02	79	8.7 ± 0.15	8	9.5 ± 0.53	53	23.2 ± 0.23
min. —								

TABLE X.
The developmental period in days at 70 per cent. R.H.

Temp. °C. and range Food	13° ± 0.5° Wholemeal flour		20° ± 0.5° Fishmeal passed 60-mesh sieve		20° ± 0.5° Flour passed 60-mesh sieve		23° ± 0.5° Dried ground carrot		27° ± 0.5° øø Wheatfeed	
	N	M ± S.E.	N	M ± S.E.	N	M ± S.E.	N	M ± S.E.	N	M ± S.E.
Larva I	125	21.6 ± 0.32	31	10.1 ± 0.23	31	10.3 ± 0.28	14	11.8 ± 0.88	10	øø7.1 ± 0.10
Larva II	107	27.6 ± 0.45	27	13.3 ± 0.33	27	9.7 ± 0.28	11	11.4 ± 0.69	10	8.8 ± 0.44
Larva III	89	80.2 ± 1.09	21	32.4 ± 1.10	26	28.6 ± 1.03	11	27.6 ± 1.50	6	25.5 ± 2.62
Larval total		139*	21	55.2 ± 1.28	26	48.4 ± 0.98	13	ø50.1 ± 2.19	6	41.0 ± 2.68
Total, egg hatched to adult emerged from cocoon	14	177.0 —	19	76.7 ± 1.3	22	70.9 ± 0.8	13	71.1 ± 2.4	—	—

* Estimated.
ø One extra instar of 12 days.
øø Kept at 20° C. and 70 per cent. R.H. for the first five days after hatching, then for the following four days the temperature rose daily from 27° to 29°C.

Large larvae, bred at 25°C., were placed in groups of eight at $-3.3^{\circ}\pm 0.8^{\circ}\text{C}$. and approximately 70 per cent. R.H. At intervals a group was removed to 13°C. and 70 per cent. R.H. for one day, then to 25°C. and 70 per cent. R.H. Four out of eight larvae survived six days exposure at $-3.3^{\circ}\pm 0.8^{\circ}\text{C}$. and became adult at 25°C. Thirteen days at -3.3°C . was lethal to all larvae. The results of this experiment support the statement of Shapiro (1941) that larvae can endure two days at 1° to 2°C. below zero.

Within the range in which development can be completed, transfer of larvae from a temperature near the optimum to one at which growth is slow, or *vice versa*, appears to have only a transitory effect on the rate of development at the final temperature. Thus when insects were started at 23°C. and transferred to 13°C. and *vice versa*, the period required to complete development at the second temperature was predictable with fair accuracy from the data for complete development at each temperature.

In general, beetles are heavier when bred at low temperatures (*cf.* Gunn & Knight, 1945). Larvae subjected to 27°C. produced light adults (Table VII), the longer the exposure to 27°C. the lighter the beetles produced. At 70 per cent. R.H., the weights of beetles bred on fishmeal at 15°C. were greater than those of beetles bred at 25°C.

	N	Female M \pm SE.	N	Male M \pm SE.
15°C. one experiment ..	33	3.65 \pm 0.04	27	3.41 \pm 0.05
25°C. heaviest result from eight experiments	15	3.25 \pm 0.33	13	3.00 \pm 0.24

Similarly on wheatfeed heavier beetles were bred from 20°C. than from 25°C. (Table XVII).

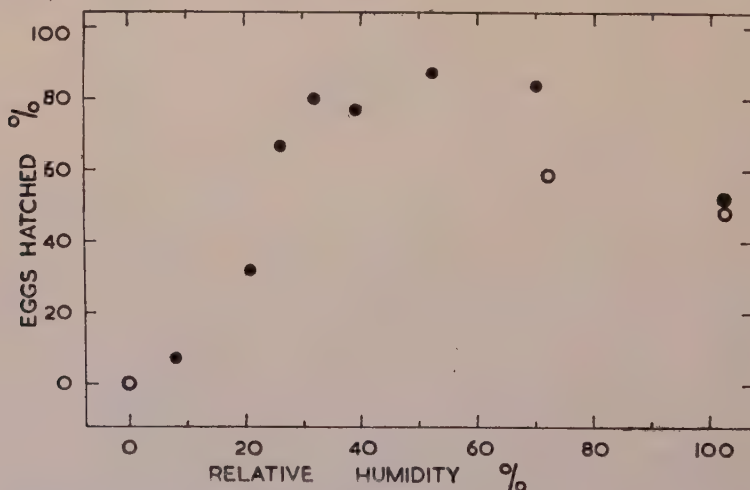
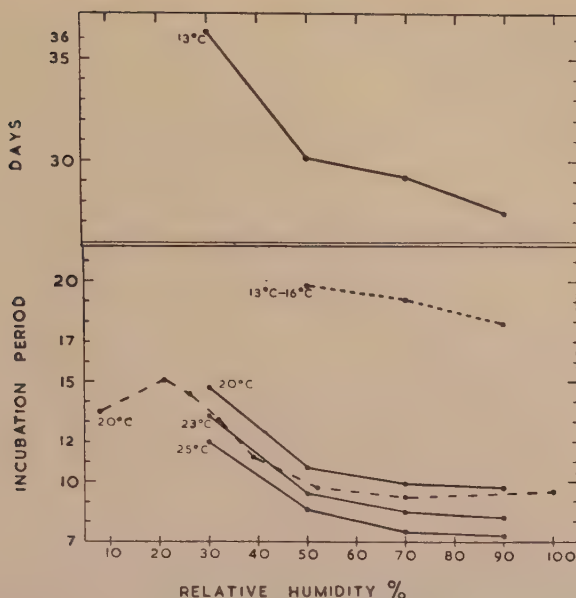


Fig. 4.—Percentage hatch of *P. tectus* eggs at 20°C., and various humidities.

Humidity.

At temperatures of 20°C. and above, humidity maintained continuously at a very high level in laboratory experiments is unfavourable for *P. tectus* larvae because the food is soon completely spoiled by moulds and as a result no larvae are able to finish development. Similar results are reported by Hickin (1942a) who found that large larvae failed to become adult at 20°C. and 90 and 100 per cent. R.H. on casein, wheat flour or rice flour. At 13°C. mould growth is lower

Fig. 5.—The egg incubation period of *P. tectus*.

than at higher temperatures and some insects are able to complete development at 100 per cent. R.H. (Table XI). The optimum humidity for rapid development is between 70 and 90 per cent. R.H. and the lowest humidity at which development can be completed is about 40 per cent. R.H. (figs. 5, 6 and 7). Fraenkel and Blewett (1943a) recorded complete development at 50 per cent. R.H., but not at 40 per cent. R.H.

TABLE XI.

Mortality and developmental periods in days on wheatfeed at 13°C. and 100% R.H.

Stage	Number of eggs or larvae used	Mortality		Developmental period days	
		No.	%	N.	M \pm S.E.
Egg	140	6	ca 5	134	19.1 \pm 0.1
Egg hatched to cocoon formation ..	30	5	17	25	72.4 \pm 2.3
Larva (including prepupal stage inside cocoon)	30	10	33	9	93.7 \pm 2.4
Pupa	29	1	3	2	16.5 \pm 0.5
Pre-emergence	27	11	37	1	53.0
Egg hatched to adult emerged ..	27	22	73	5	180.6 \pm 5.7

One larva accidentally killed inside the cocoon. Two adults removed *alive* from complete cocoons. Only 30 of 134 eggs hatched used for experiment. The numbers of insects available for determining developmental periods after cocoon formation are small due to the building of many cocoons away from the glass wall of the tubes.

The results of an experiment to determine egg mortality at various humidities are shown in fig. 4. The eggs were laid at 20°C. and 70 per cent. R.H. over one day and transferred to the experimental humidities immediately following this oviposition period. At each humidity 60 to 100 eggs were incubated in groups of 20 per 2 \times 1 inch tube. Hatching at favourable humidities (70 per cent. R.H.) is normally above 80 per cent. At higher humidities, especially at 100 per cent. R.H., moulds grew quickly, and since the eggs used were closely bunched together it was thought that this might have favoured mould growth and inhibited hatching. The experiments at this humidity and at 70 per cent. R.H. were

repeated, therefore, with the eggs more widely spaced (circles in fig. 4), but mould growth, though less, was still extensive and about the same proportion of eggs hatched at 100 per cent. R.H. These eggs were laid by a different batch of beetles and there was a much poorer hatch in the control at 70 per cent. R.H., so it can be inferred that the slight reduction of mould growth did slightly improve the hatching. Although at 90 per cent. R.H. mould growth was usually extensive, egg mortality was seldom much greater than at 70 per cent. R.H. At 30 per cent. R.H. egg mortality was often much greater than in the experiment figured, normally only about one-third of the eggs hatching.

For several temperatures and humidities the incubation periods of eggs, which were laid at 70 per cent. R.H. at the experimental temperatures over a period of 24 hours, and then placed at the experimental humidities, are shown in fig. 5. The mean periods are based on 30 to 160 eggs hatched, except at 8 per cent. R.H., under which conditions only 6 eggs hatched. Humidities below 50 per cent. R.H. retarded hatching. At low humidities the mandibles were visible for a considerable time before hatching, implying that retardation of hatching is due to difficulty of emergence rather than to slow development. This is illustrated by the following results at 20°C.

	Eggs used	Eggs hatched	Incubation period (days) M \pm S.E.
Continuously at 8% R.H.	80	6	13.5 \pm 0.8
10 days at 8% R.H., then transferred to 70% R.H.	20	13	11.1 \pm 0.1
Continuously at 70% R.H.	80	68	9.2 \pm 0.1

It will be seen that eggs hatched very soon after transfer to a favourable humidity, whereas the few that hatched at 8 per cent. R.H. had a considerably longer incubation period. Ewer and Ewer (1942) interchanged eggs between high and low humidities and showed that the earlier stages of embryonic development were not sensitive to low humidity. They suggest that the effect of low humidity on the later stage of embryonic development could be due to a slowing up of development, to a depression of the activity of the emerging larvae, or to hardening of the egg shell.

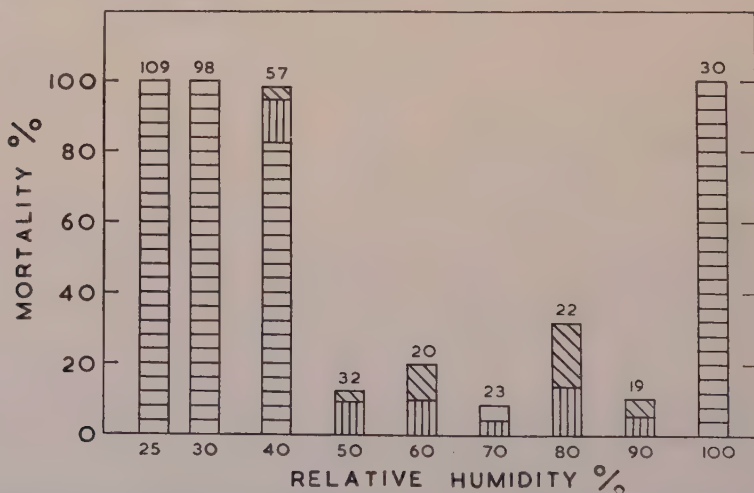


Fig. 6.—Percentage of *P. tectus* dying (at 25°C. on fishmeal) in the various post-oval stages at various humidities. Shading represents mortality in first larval instar (horizontal), second and third (vertical), and in pupal stage (oblique). No shading (at 70 per cent. R.H. only) represents mortality of adult in cocoon. The numbers above the histograms indicate the number of insects tested at each humidity.

Experiments were carried out to ascertain the effect on various post-oval stages of various humidities (fig. 6). Eggs were hatched at 70 per cent. R.H. for humidities of 40 per cent. R.H. and above, and at the experimental humidity for the lower humidities. These experiments showed that there is little difference in larval mortality at humidities between 50 and 90 per cent. R.H., but at 40 per cent. R.H. and below and at 100 per cent. R.H. most larvae died in the first instar.

The effect of humidity on the length of the developmental period of larvae from eggs hatched at 70 per cent. R.H. is illustrated by fig. 7. In another experiment at 40 per cent. R.H., the eggs were incubated at 40 per cent. R.H., but this made little difference to the larval period.

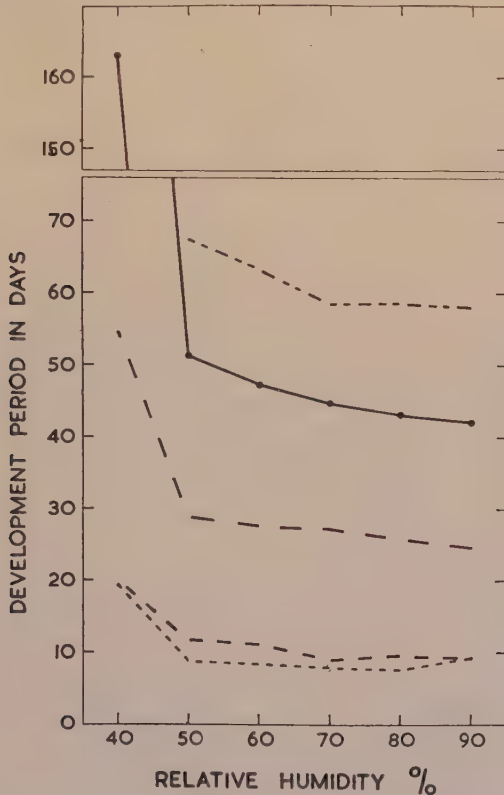


Fig. 7.—Development after egg-hatching of *P. tectus* on fishmeal at 25°C. and different humidities. The curves from bottom to top represent larva I, larva II, larva III, total larva, egg hatched to adult emerged.

Similarly, the effect of humidity on the pupal period at two temperatures is shown in fig. 8. At 20°C. the insects were bred at 70 per cent. R.H. and transferred to the experimental humidity on the day of pupation. At 25°C. the insects were bred at the experimental humidity from the hatching of the egg.

Further experiments were carried out for the effects of humidity on the pre-emergence period and mean weight of adult beetles. For the former (fig. 9), insects tested at 25°C. were bred at the experimental humidity from the hatching of the egg. At 20°C. the insects were bred at 70 per cent. R.H., large larvae from cultures being placed singly in $2 \times \frac{1}{2}$ inch tubes, and transferred to the experimental humidity on the day of pupation. Two experiments were carried

out at this temperature with insects from different cultures. The results of these experiments relating the pre-emergence period to humidity can be expressed by straight regression lines with slopes significantly different from zero. At 20°C . the values of the regression coefficient b are -0.46 and -0.26 and at 25°C . the value of b is $+0.28$. The shortening of the pre-emergence period when the larva is bred at low humidities is attributable to the weak poorly made cocoons constructed at low humidities.

In the experiments to determine the effect of humidity on the mean weight of adults (fig. 10), insects tested at 25°C . were bred on fishmeal at the experimental

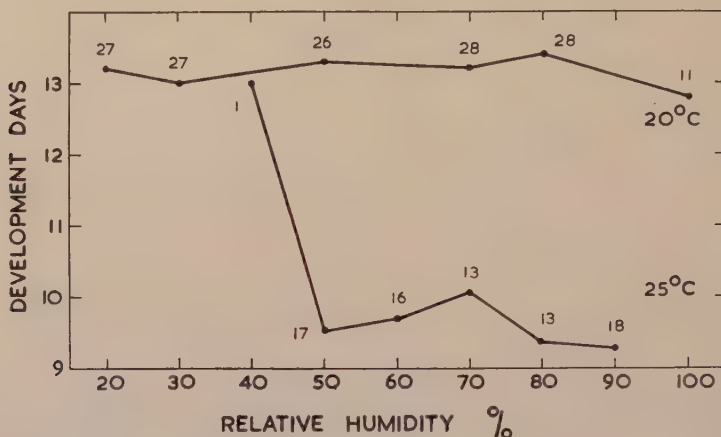


Fig. 8.—The pupal period at different humidities and two temperatures. The numbers completing the pupal period at each condition are shown.

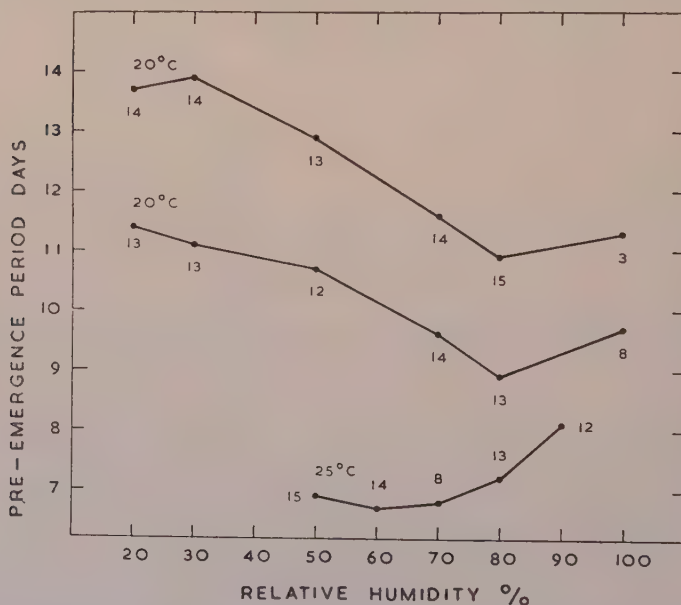


Fig. 9.—Pre-emergence period at different humidities and two temperatures. The numbers on which the periods are based are given near each point.

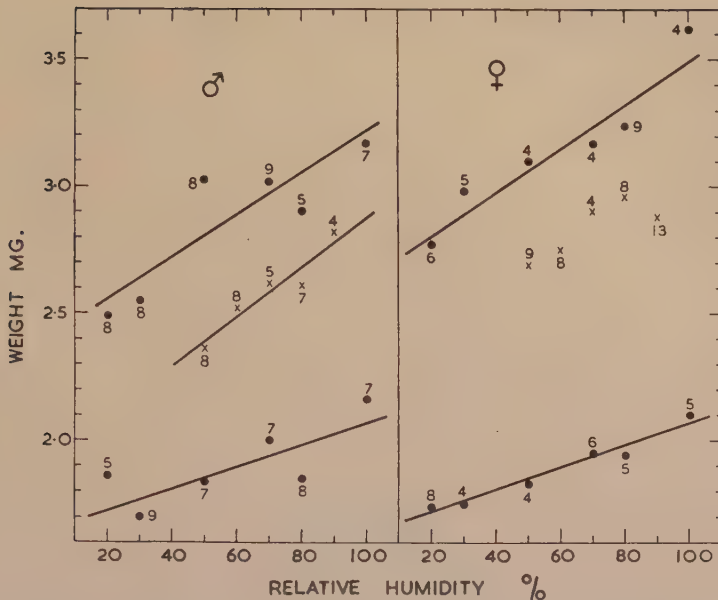


Fig. 10.—The mean weight of adult *P. tectus* at several humidities and two temperatures.

Crosses indicate experiments at 25°C. and dots indicate those at 20°C.

All the regression lines given differ significantly from zero and the values of b are, top to bottom: ♂♂, 0.083, 0.098, 0.043; ♀♀, 0.086, regression insignificant and line omitted, 0.044. The numbers given are the numbers of beetles on which the means are based.

humidities. At 20°C. insects were bred on wheatfeed at 70 per cent. R.H. until pupation, and then transferred to the experimental humidities. At this temperature two groups of experiments were done; the larvae yielding adults of the higher mean weight were taken from a less crowded culture than the other. The relationship between beetle weight and experimental humidity can be represented by straight regression lines (given in fig. 10) with a coefficient b significantly different from zero for both sexes at 20°C. but only for the male at 25°C.

Prolonged low humidity is obviously unfavourable, especially when below 50 per cent. R.H. In addition to increasing mortality, the developmental period of the larva is increased and the insects bred, if any, may be smaller. The developmental period of the pupa is generally very stable, and is only affected if the larva has been subject to a prolonged unfavourable humidity.

The effects of low humidity in the experiments described here can be summarised as follows:—

		Insects kept continuously at low R.H. from hatching 25°C.	Insects kept at low R.H. after pupation 20°C.
Developmental period of pupa ..	(fig. 8)	increased	unaffected
Pre-emergence period	(fig. 9)	decreased	increased
Larval and total developmental period	(fig. 7)	increased	—
Weight of adult	(fig. 10)	decreased	decreased

Ewer and Ewer (1942) state that larvae eat less at low than at high humidities. This may help to explain the slow development, and also the low weight of beetles grown at low humidities. Ewer and Ewer also state that the pre-emergence period is unaffected by low humidity.

An attempt to separate the effects of food moisture content from those of the humidity of the air did not succeed. Newly-hatched larvae were placed on a hygroscopic food, dried onion, at low humidity (30 per cent.) but the food became a sticky lump in which the larvae were unable to develop.

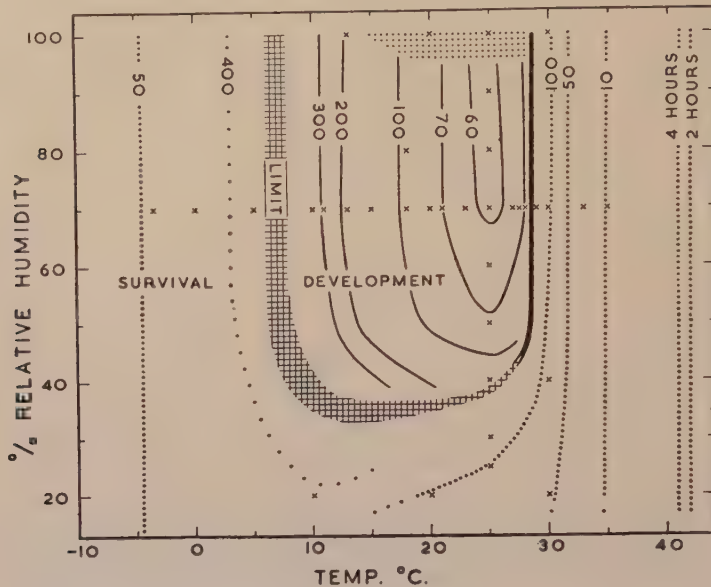


Fig. 11.—Developmental periods and physical limits for *Ptinus tectus*.

Limiting temperature—humidity combinations.

The foregoing sections on the effects of temperature and humidity on development can be summarised briefly. At favourable humidities eggs hatch over a temperature range of 30° to 10°C., and larvae and pupae will develop between 28° and 5°C. At moderate temperatures mould may prevent development at high humidities but otherwise high humidity is favourable. At relative humidities below 40 per cent. egg hatching is erratic, and larval and pupal development retarded. If such low humidities persist for long, larvae die, especially in the younger stages.

The combined effects of temperature and humidity are shown graphically in fig. 11. In this figure, crosses mark the physical conditions at which experiments have been done, using numbers of insects kept singly in glass tubes, on wheat-feed. The full lines represent, in days, the mean complete developmental period from laying of the egg to emergence of the adult from the cocoon. The dotted lines are based on the periods of exposure survived by the most resistant insects used. The line marked "limit" gives the limiting physical conditions for complete development. The detailed position of this line is more precisely determined for the higher temperatures. The stippled area shows conditions in which the completion of development is prevented by extensive mould growth.

Some additional experiments were performed to determine the resistance of various stages to unfavourable combinations of temperature and humidity. Egg survival in these experiments is given in Table IV. In experiments in which the eggs were left to hatch or die, some hatching was observed at low humidities or extreme temperatures if the other condition was favourable. In experiments in which both temperature and humidity were unfavourable, the number of eggs placed at each condition was small but the overall results were consistent. At

30°C. and 20 per cent. R.H., eggs were killed by exposure for between five and seven days. At 10°C. and 20 per cent. R.H. all eggs died when exposed between 41 and 53 days.

TABLE XII.

Reaction of larvae, 22 to 23 days old at 25°C. and 70% R.H. to various combinations of temperature and humidity.

Temp. °C.	R.H. %	No. of larvae exposed	% emerged as adults	Time from exposure to death in days		Time from exposure to pupation in days		
				M ± S.E.	Range	N	M ± S.E.	
35	70	21	0	7.8±0.2	6-9	—	—	
33	70	19	0	11.5±1.0	8-22	—	—	
30	20	16	0	29.8±3.4	6-51	—	—	
30	40	20	0	29.6±3.4	5-61	—	—	
30	70	20	5	49.8±5.3	19-80	1	14	
25	70	20	95	—	—	20	11.3±0.3	
20	20	20	70	—	—	20	22.1±0.5	
10	20	17	71	—	—	16	210.6±8.0	
10	70	16	100	—	—	16	124.8±8.7	
5	70	20	50♂	—	—	8	275.9±8.1	

♂ To time of the appearance of adults in cocoons only.

TABLE XIII.

Reaction of pupae exposed to various combinations of temperature and humidity 24 to 48 hours after pupation at 25°C. and 70% R.H.

(Only 7 pupae used at each condition.)

Temp. °C.	R.H. %	% emerged as adults	Period in days from beginning of exposure to death			Period in days from pupation to emergence from cocoon	
			N	M	Range	N	M
35	70	0	7	10.0	8-12	—	—
33	70	0	7	12.1	9-16	—	—
30	20	43	♂2	9.5	9-10	3	17.0
30	40	0	♂—	—	—	—	—
30	70	86	1	26	—	6	24.5
25	70	100	—	—	—	7	19.1
20	20	86	1	21	—	6	26.5
10	20	0	♂3	9.3	7-11	—	—
10	70	100	—	—	—	♂♂7	92.3
5	70	0	7	66.7	14-92	—	—

♂ Complete records of date of death not kept.

♂♂ Pupa 66.9 days, pre-emergence 25.4 days.

TABLE XIV.

Duration in days of post-larval periods at various combinations of temperature and humidity for experiment given in Table XII.

Temp. °C.	R.H. %	Pupa		Pre-emergence		Pupa & pre-emergence	
		N	M ± S.E.	N	M ± S.E.	N	M ± S.E.
30	70	—	—	—	—	1	18.0
25	70	—	—	—	—	20	20.7±1.1
20	20	—	—	—	—	15	19.6±0.6
10	20	12	32.3±4.3	11	27.2±3.5	—	—
10	70	15	41.7±4.5	15	35.2±2.8	—	—
5	70	6	75.8±8.8	1	30.0	—	—

At favourable humidities, even third instar larvae died at 30°C. (Table XII), only one pupating at 70 per cent. R.H., but some pupae survived this temperature even at 20 per cent. R.H. (Table XIII). Pupae, on transfer from 25°C. and 70 per cent. R.H. survived at 10°C. and 70 per cent. R.H., but died at both 10°C. and 20 per cent. R.H., and 5°C. and 70 per cent. R.H. (Table XIII). If transferred to these temperatures as large larvae, however, some larvae survived to pupate (Table XII) and the majority of these pupae became adult (Table XIV). At 10°C. larval mortality was slight, but it was severe at 5°C., at which temperature growth was very slow so that few insects became adult (Table XII).

Food.

Experiments to compare the development of *P. tectus* on different foods were first performed using either groups of newly-hatched larvae, 5 or 10 in a 2 × 1 inch tube, or jam jar cultures of adults producing variable numbers of offspring. Results in these experiments were often unsatisfactorily variable, being subject to the group effect mentioned by Gunn and Knight (1945). These authors noted that individuals of this species bred in groups were lighter in weight and grew more slowly than other individuals bred singly. Dense crowding caused more obvious effects. In tubes (Table XV) the degree of crowding could not be controlled because mortality differed from tube to tube, whilst in cultures the numbers of eggs produced also differed. Consequently in later experiments, larvae were bred singly in 2 × $\frac{1}{2}$ inch tubes with an excess of food (Tables XVII to XXI). In these, results were less variable.

Some of the experiments grouped in Table XV were infested by the mite, *Glycyphagus destructor* (Schr.). Although this is a débris feeder, it almost certainly retarded development in some instances, and appears to have caused some deaths. Thus, by delaying pupation, it tended to increase the mean larval periods, and conversely by killing off the slower growing larvae it decreased these means.

Two criteria for comparing foods are used, the developmental period of the larvae and the weight of the adult beetles produced on a food. Sometimes in place of the larval period it has proved more convenient to use the period from egg hatching to emergence of the adult from the cocoon, as for instance when pupation inside the cocoon cannot be observed or disturbance of the experiment needs to be minimal. These measures are approximately identical because the pupal and pre-emergence periods remain very constant on different foods in the same physical conditions. The larval period is probably a better criterion than the initial adult weight for the comparison of different foodstuffs for *P. tectus*, because the adult feeds regularly and does not need to rely on stored food for the production of eggs. Beetle weight appears to be less consistent than the larval period. It depends on sex and also starts to decrease as soon as the beetle emerges from the cocoon, so that it is necessary to weigh the beetle as early as possible after emergence. Nevertheless, it shows clearly differences between foodstuffs. Thus in an experiment in which offspring of a group of beetles were bred in four cultures for each of four foodstuffs at 25°C. and 70 per cent. R.H., the mean weights in milligrams (with the number of beetles in parentheses) were as follows:—fishmeal, 3.43 (644); yeast powder, 3.31 (451); wholemeal flour, 2.51 (386); soya bean meal (1.4 per cent. fat), 2.40 (147).

Both weight and larval period are shown for a number of foodstuffs in Table XVII. It is clear from this Table that the order of suitability of foodstuffs for body weight is different from that for rapid development but there are no gross discrepancies. High body weight is usually obtained from foods of high protein content. Foods yielding very light adults usually allow only slow development.

TABLE XV.

Period in days required for complete development from the hatching of the egg to the emergence of the adult from the cocoon when bred in groups on various foods at 23°C. and 70% R.H. in 2 × 1 inch glass tubes.

No.	Food	No. of Insects			Development period M ± S.E.	Significance of comparison (P.)
		Per tube	Total used	Emerged		
1	Beef	10	40	0	—	
2	Beef and carrot mixture ..	10	20	7	80 ± 2.3	
3	Beef and carrot mixture, ground	10	20	0	—	
4	Beetroot	10	20	0	—	
5	Beetroot, ground	10	20	0	—	
6	Beetroot, ground + yeast ..	5	20	ø 2	81 ± 11.0	
7	Cabbage	10	20	10	78 ± 3.2	7 cf 8 = 0.1
8	Cabbage, ground	10	20	15	72 ± 0.7	8 cf 9 < 0.001
9	Cabbage, ground + yeast ..	5	20	ø 15	59 ± 0.5	
10	Carrot	10 & 5	40	15	94 ± 1.5	
11	Carrot, ground	10	10	5	98 ± 0.7	11 cf 12 < 0.001
12	Carrot, ground	10	10	8	77 ± 4.9	12 cf 13 = 0.1
13	Carrot, ground + yeast ..	5	20	ø 13	67 ± 2.4	11 cf 13 < 0.001
14	Egg	10	50	8	132 ± 2.6	14 cf 15 = 0.001
15	Egg	5	10	ø 2	113 ± 3.0	15 cf 16 < 0.001
16	Egg	5	10	ø 4	73 ± 1.0	16 cf 17 = 0.02
17	Egg + yeast	5	20	ø 4	61 ± 2.8	15 cf 17 < 0.001
18	Fishmeal	5	10	ø 10	73 ± 1.2	18 cf 19 = 0.1
19	Fishmeal	10	20	12	70 ± 0.9	18 + 19 cf 20 = 0.001
20	Fishmeal	5	25	ø 13	65 ± 0.9	20 cf 21 = 0.001
21	Fishmeal	5	25	ø 14	61 ± 1.0	21 cf 22 = 0.001
22	Fishmeal + yeast	5	20	ø 13	57 ± 0.3	
23	Herring-meal	10	40	0	—	
24	Herring-meal + yeast	5	20	ø 0	—	
25	Milk powder	10	30	14	104 ± 3.8	25 cf 26 < 0.001
26	Milk powder	5	20	ø 8	78 ± 1.0	
27	Milk powder + yeast	5	20	ø 1	56	
28	Onion	10	20	1	188	
29	Onion, ground	10	20	2	149 ± 20.5	29 cf 30 = 0.05
30	Onion, ground + yeast ..	5	20	ø 3	89 ± 3.0	
31	Potato	10	20	0	—	
32	Potato, ground	10	10	1	106	
33	Potato, ground	10	30	21	69 ± 1.0	33 cf 34 < 0.001
34	Potato, ground + yeast ..	5	20	17	5 ± 91.2	
35	Swede	10	10	3	98 ± 1.2	
36	Swede, ground	10 & 5	15	ø 6	91 ± 2.4	36 cf 37 = 0.001
37	Swede, ground	5	5	ø 5	82 ± 0.2	37 cf 38 = 0.1
38	Swede, ground	10	10	6	76 ± 1.5	
39	Swede, ground + yeast ..	5	5	ø 4	57 ± 0.5	39 cf 40 = 0.05
40	Swede, ground + yeast ..	5	5	ø 4	64 ± 3.7	37 + 38 cf 39 + 40 < 0.001
41	Wheat, undamaged	10	10	5	86 ± 0.8	41 cf 42 < 0.001
42	Wheat, undamaged	10	10	5	73 ± 1.2	
43	Wheat, damaged	10	20	14	93 ± 1.3	
44	Wholemeal flour	10 & 5	20	ø 17	80 ± 1.0	44 cf 45 < 0.001
45	Wholemeal flour	10	10	10	73 ± 0.8	45 cf 46 = 0.05
46	Wholemeal flour	5	5	ø 3	67 ± 2.5	46 cf 47 = 0.02
47	Wholemeal flour	5	5	ø 2	55 ± 0.0	
48	Yeast powder	5	10	ø 7	64 ± 1.5	48 cf 49 = 0.02
49	Yeast powder	5	10	ø 7	59 ± 0.6	

ø Became infested by *Glycyphagus destructor*.

1. Natural foods.

The series of foods compared included produce normally found in warehouses and shops and also a number of wartime dehydrated vegetables and meats normally marketed in tins. The vegetables were available in strips and the meats in greasy lumps. When possible, samples of each were ground to a powder and the food tested in both states. The composition of the foods is given by Howe and Burgess (1952).

Results with larvae bred in groups are presented in Table XV and some similar unpublished results of Souter * using ground foodstuffs are given in Table XVI. Results with larvae bred singly are given in Tables XVII to XX. With lumpy foods disturbance of tubes during examination may break the cocoons, prolong the post-feeding larval period and cause some mortality. Newly-hatched larvae have some difficulty in attacking hard foods like dehydrated strips of potato. *P. tectus* could not develop on the very greasy beef or herring meal, even when these were diluted by half with ordinary fishmeal (Table XVII) or with yeast added (Table XV). Development was also poor with sticky sugary foods such as beet and onion, and unground hard foods like potato (Table XV).

Fraenkel and Blewett (1943a) give diagrams representing the larval plus pupal period of *P. tectus* on cereal products and other dried foodstuffs. The median period of development obtained from their figures is given in Table XVI, which, allowing about 10 days for the pupal period, indicates rather slower development than that in Tables XVII and XIX.

TABLE XVI.

Developmental period in days of *P. tectus* bred in groups at 25°C. and 70% R.H. on ground dehydrated foods (from Souter, 1945, unpublished report) and on dry foodstuffs (from Fraenkel & Blewett, 1943a).

SOUTER
5 larvae per tube, 15 used per food.

FRAENKEL & BLEWETT
10 larvae per tube, 20 used per food.

Food	% pupated	Mean larval period	Food	% pupated	Median period, larva + pupa
Beef ..	0	—	(their fig. 5)		
Beetroot ..	0	—	Wholemeal flour ..	65	61
Cabbage ..	86	53.5	85% flour ..	70	60
Carrot ..	27	58	N.S.R. 73% flour ..	25	108
Egg ..	27	86	Patent flour ..	10	95
Herring ..	0	—	Bran ..	80	56.5
Milk ..	40	80	Middlings ..	50	65
Onion ..	34	173.5	Weatings ..	60	62
Potato ..	27	87	Wheatgerm ..	60	68
Swede ..	27	58	(their fig. 16)		
			Dead insects..	75	45
			Dried milk ..	45	93
			Fishmeal ..	40	64.5
			Meat meal ..	75	56.5
			Pea flour ..	25	88.5
			Wholemeal flour ..	65	61.5
			Yeast ..	75	56

Wheatfeed is the best of the three usual foods employed in this work, development proceeding more quickly than on either fishmeal or wholemeal flour. The results are shown in fig. 12 of experiments simultaneously performed in which larvae were bred singly in $2 \times \frac{1}{2}$ inch tubes at 25°C. and 70 per cent. R.H. In

* Souter, E. C. (1945). Insect attack on dehydrated foods. Unpublished Report, Dep.Sci.industr. Res., U.K., Pest Infestation Laboratory.

TABLE XVII.
Developmental period in days and weight of adult beetles at emergence from cocoons
bred singly on various foods at 70° R.H. and various temperatures.

Food	% died	Developmental period in days						Weight in mg.					
		Larva			Egg hatched to adult emerged			Females		Males			
		N	M	S.E.	N	M	S.E.	N	M ± S.E.	N	M ± S.E.		
25° C.													
Wheatfeed ..	12	18	32	± 1.0	18	49	± 1.0	8	3.36 ± 0.07	10	2.95 ± 0.06		
Synthetic diet ..	13	29	35	0.7	—	—	—	—	—	—	—		
Wheat germ ..	17	26	36	0.4	—	—	—	—	—	—	—		
Soyameal, 1.4% fat ..	10	27	38	0.2	27	57	± 0.2	13	2.39 ± 0.07	12	2.37 ± 0.04		
Grassmeal ..	18	11	39	0.4	11	56	1.3	5	2.52 ± 0.14	7	2.50 ± 0.12		
Fishmeal and yeast ..	5	57	39	0.3	51	60	0.3	30	3.81 ± 0.07	23	3.43 ± 0.05		
Wholemeal flour ..	6	28	40	0.5	25	58	± 0.6	14	2.61 ± 0.08	13	2.38 ± 0.02		
Yeast powder ..	0	30	40	0.5	21	58	± 1.0	11	3.14 ± 0.05	9	2.92 ± 0.07		
Dust I ..	15	17	40	0.8	17	56	0.8	13	2.72 ± 0.07	3	2.00 ± 0.10		
Dust II ..	5	18	40	1.1	19	55	± 0.2	9	2.03 ± 0.08	10	1.98 ± 0.04		
Fishmeal ..	10	27	43	± 0.5	22	60	± 0.8	16	3.16 ± 0.03	7	2.94 ± 0.06		
Soyameal, 7.2% fat ..	24	20	47	± 1.3	—	—	—	—	—	—	—		
Wholemeal ..	30	9	49	4.1	10	66	± 2.3	12	3.02 ± 0.57	1	3.00		
Rat droppings ..	70	2	69	6.5	6	89	4.2	2	2.00 ± 0.00	4	1.49 ± 0.19		
23° C.													
Wheatfeed ..	10	36	34	0.6	36	54	± 0.2	21	3.14 ± 0.06	15	2.93 ± 0.05		
Artox flour ..	12	59	35	1.1	48	55	± 0.3	24	3.43 ± 0.06	19	3.13 ± 0.06		
Fishmeal ..	10	19	52	1.3	17	68	± 1.0	8	3.01 ± 0.12	10	2.64 ± 0.13		
Fishmeal, herring meal 3:1 ..	10	17	67	± 1.8	17	87	± 2.0	10	3.51 ± 0.11	6	2.92 ± 0.24		
Fishmeal, herring meal 2:2 ..	100	—	—	—	—	—	—	—	—	—	—		
Fishmeal, herring meal 1:3 ..	100	—	—	—	—	—	—	—	—	—	—		
Cotton seed meal ..	28	50	57	± 1.0	50	78	± 1.0	20	2.42 ± 0.08	28	2.01 ± 0.06		
Soya-bean meal, 1.4% fat ..	ø	13	69	4.0	12	90	4.5	8	1.72 ± 0.14	5	1.50 ± 0.17		
Soya-bean meal, 7.2% fat ..	65	10	75	2.3	5	94	4.0	1	1.80	4	2.72 ± 0.25		
20° C.													
Wheatfeed ..	0	48	39	0.3	49	63	± 0.3	22	3.62 ± 0.04	27	3.30 ± 0.03		
Artox flour ..	+50	33	42	0.4	15	63	± 0.6	8	3.09 ± 0.15	8	2.76 ± 0.11		
Wheatfeed ..	20	32	43	0.3	32	66	± 0.3	12	3.48 ± 0.25	20	3.10 ± 0.04		
Yeast powder ..	0	20	48	0.7	19	74	± 0.8	10	3.60 ± 0.03	9	3.14 ± 0.09		
13° C.													
Wheatfeed ..	15	10	98	—	10	152	—	—	—	—	—		
Wholemeal flour ..	15	105	129	—	105	177	—	—	—	—	—		
Canadian white flour ..	4	150	139	± 1.9	145	185	± 2.0	66	3.06 ± 0.02	71	2.79 ± 0.02		
Copra meal ..	50	16	156	—	16	209	—	—	—	—	—		

ø A few Psocids found in some tubes.

+ High mortality due to *Glyciphagus destructor*.

LARVA. TOTAL.	
W	$32.6 \pm 0.2 (23) \quad 51.0 \pm 0.4 (24)$
W+Y	$34.5 \pm 0.5 (23) \quad 52.3 \pm 0.5 (23)$
Fl	$45.9 \pm 5.8 (20) \quad 64.3 \pm 5.8 (21)$
Fl+Y	$34.0 \pm 0.5 (22) \quad 51.7 \pm 0.6 (23)$
F	$41.5 \pm 0.9 (22) \quad 60.3 \pm 0.9 (20)$
F+Y	$34.9 \pm 0.9 (21) \quad 53.6 \pm 1.3 (23)$

Fig. 12.—The larval period in days at 25°C., 70 per cent. R.H., and the total developmental period from egg hatching to the emergence of the adult from the cocoon for *P. tectus* in concurrent experiments with wheatfeed (W), wholemeal flour (Fl) and fishmeal (F) with and without yeast (Y) added.

this figure the lengths of the lines are proportional to the respective means which are given over each line, together with the standard error and, in brackets, the number of insects on which these figures are based. The adults produced are also heavier than those bred on flour and as heavy as those from fishmeal (Table XVIII). The addition of five per cent. by weight of yeast to wheatfeed did not improve it but a similar addition to fishmeal and to wholemeal flour increased the developmental speed for both and the weight for flour. Wheatgerm, dehydrated potato ground very fine (Table XIX) and Artox flour (Table XVII) were the only other ordinary foods to give larval development about as fast as wheatfeed although many foods did so when five per cent. by weight of dried yeast was added. Development, however, on yeast powder alone was slower than on wheatfeed (Table XVII). The adults produced were as heavy as those obtained from fishmeal.

TABLE XVIII.
Weight of adults at emergence from cocoons (in mg.).

Food	Weight of ♀♀		Weight of ♂♂		Developmental Mortality %
	N	M ± S.E.	N	M ± S.E.	
Wheatfeed	13	3.42 ± 0.10	11	3.12 ± 0.08	4
Wheatfeed and yeast ..	12	3.33 ± 0.09	10	3.13 ± 0.15	4
Fishmeal	11	3.48 ± 0.07	9	3.14 ± 0.09	16
Fishmeal and yeast ..	13	3.49 ± 0.10	9	3.11 ± 0.08	8
Wholemeal flour	14	2.73 ± 0.06	5	2.46 ± 0.05	16
Wholemeal flour and yeast	10	3.33 ± 0.13	13	3.02 ± 0.07	4

Periods of development of these beetles are shown in fig. 12.
For both sexes the only significant differences are those between flour and all other foods.

Fraenkel and Blewett (1943a) found that the only cereal derivative superior to wholemeal flour was bran, while products such as middlings and weatings, apparently similar to wheatfeed, gave poorer results of the same order as high extraction flours. These results are unexpected and those of the present work are more usual. The bodies of dead insects was the only food used by Fraenkel and Blewett that gave results as good as the wheatfeed used in this work.

TABLE XIX.

Length of larval life in days at 25°C. and 70% R.H. on various foods ground and sieved to pass a number of sieves.

Food	Sieves used, Meshes per inch	Coarsest		Medium		Finest	
		N	M \pm S.E.	N	M \pm S.E.	N.	M \pm S.E.
Dried carrot ..	30 & 50	10	77.1 \pm 3.7	12	88.2 \pm 3.3	18	60.2 \pm 8.6
Fishmeal ..	50 & 70	26	45.3 \pm 4.7	28	46.8 \pm 7.1	30	45.4 \pm 4.4
Dried potato ..	36 & 50	1	80 —	0	— —	24	35.0 \pm 0.3
Soya meal ..	30 & 50	18	68.1 \pm 4.1	25	54.6 \pm 2.3	30	38.6 \pm 0.5
Yeast powder	50 & 70	26	38.9 \pm 3.4	28	38.6 \pm 2.8	27	38.3 \pm 3.0
Wholemeal flour	30 & 50	29	42.7 \pm 5.2	28	42.4 \pm 5.3	28	45.7 \pm 7.3
Wholemeal flour	10, 16 & 30	26	36.6 \pm 1.8	29	37.3 \pm 2.6	29	37.2 \pm 2.4

Thirty larvae used for each fraction.

φ Entire wheat ground to pass each sieve, so that all samples contain the entire grain.

The particle size of homogeneous foods affects the rate of development (Howe, 1949b), but with some foods such as wholemeal flour, grinding and sieving into fractions passing various sieves produces foods with different food values. Thus (Table XIX) finely divided soya meal was more easily utilised than coarser material but, with wholemeal flour, development was quickest on the coarser fractions as these contained more bran than the finer portions. When flour was ground to pass various meshes without rejecting any of the whole grain the particle size did not affect development.

Development proceeded rapidly on two samples of warehouse dust (Table XVII). These were taken from the cavity between the double walls of a grain warehouse and passed through a sieve of 30 meshes to the inch to remove the grain and rat droppings. The development was quicker than on fishmeal but much slower than on wheatfeed (Table XVII). Development was also possible in rat droppings, but was slow, most of the larvae pupating inside the pellets. In warehouses some *Ptinids* do breed in the rat droppings. In recent years it has been assumed that these faecal pellets could be an important source of food, but if the results obtained here are typical, their importance has been overrated.

Since young larvae are generally more susceptible to adverse conditions than older ones, it was thought that deficiencies in poorer foodstuffs might act more severely on younger larvae. Therefore an experiment was performed in which larvae were set up at 25°C. and 70 per cent. R.H. on both wheatfeed and fishmeal. After ten days some larvae from each food were transferred to the other food and the rest left undisturbed on their original food. In addition a number of larvae were bred through on a mixture of wheatfeed with 10 per cent. by volume of fishmeal. Development was slightly accelerated on this mixture (Table XX). Giving fishmeal to young larvae fed later on wheatfeed caused no change as

TABLE XX.

Larval period in days of *P. tectus* bred singly at 25°C. and 70% R.H.

	N	M \pm S.E.	% died
(a) Wheatfeed	23	32.6 \pm 0.2	0
(b) Wheatfeed + 10% fishmeal	23	31.7 \pm 0.4	4
(c) Fishmeal for 10 days then wheatfeed	24	32.5 \pm 0.5	4
(d) Wheatfeed 10 days then fishmeal	21	34.8 \pm 0.5	4
(e) Fishmeal	22	41.5 \pm 0.9	12

Differences are significant except for (c) as compared with (b) and (a) as compared with (c).

TABLE XXI.

The larval period in days of *P. tectus* bred singly at 20°C. and 70% R.H. on synthetic diets.

Food			N	M \pm S.E.	Mortality (% died)
Parts casein	Parts glucose				
50	50	without sterol	36	47.6 \pm 0.7	21
100	0	with sterol	44	46.4 \pm 0.6	13
75	25	" "	39	44.6 \pm 1.0	12
50	50	" "	38	43.9 \pm 0.6	13
25	75	" "	39	45.2 \pm 0.7	16
10	90	" "	36	45.7 \pm 0.7	17

Means were significantly different in the following:—

- 50 parts casein with sterol, as compared with 50 parts casein without sterol ($P=0.01$).
 75 parts casein with sterol, as compared with 50 parts casein without sterol ($P=0.05$).
 25 parts casein with sterol, as compared with 50 parts casein without sterol ($P=0.02$).
 50 parts casein with sterol, as compared with 100 parts casein with sterol ($P=0.001$).

compared with wheatfeed given continuously, but larvae fed on wheatfeed for ten days and then transferred to fishmeal developed more slowly. Both groups of transferred larvae grew more quickly than would be expected from the developmental periods on each food alone, and growth was accelerated by the mixed foodstuffs, so it appears likely that each food has some deficiency which is made up by the other. Certainly young larvae were not more affected than older ones.

2. Synthetic foods.

Fraenkel and Blewett (1943b) developed an artificial diet of casein, glucose, dried brewers' yeast (five parts), cholesterol (one part) and McCollum's mineral salt mixture (two parts), on which *P. tectus* grows almost as rapidly as on wheatfeed (Tables XVII and XXI). The ratio of casein to glucose in this diet was varied to give five diets differing only in the proportion of protein and carbohydrate (Table XXI). A sixth diet was also used in which the sterol was omitted.

The omission of the sterol significantly slowed development, showing that the sterols supplied by the yeast are insufficient for rapid optimal development. The only other diet on which the growth rate was significantly retarded was that with no glucose. The most rapid development was obtained on the diet with equal parts of glucose and casein and with sterol added.

Dr. D. L. Gunn (unpublished private correspondence) found that the percentage of starch in the faeces taken from a culture of *P. tectus* was greater than that in the wholemeal flour used as food, suggesting that a large quantity of food was passed through the gut to obtain sufficient protein.

3. Conclusions.

These experiments confirm the conclusions of Fraenkel and Blewett (1943a) that *P. tectus* can live on a diet substantially free of carbohydrate, but they also indicate that faster development is obtained with an adequate quantity of carbohydrate available. In contrast to the findings of Fraenkel and Blewett, faster development was obtained on some vegetable foods than on most animal foods or on yeast. These authors also remark that *P. tectus* thrives well on foods of high fat content such as dried insects, and better than many other insects on dried milk in spite of a vitamin deficiency in this food. In our experience, however, a high fat content is of no advantage and none of the fatty foods tried proved completely satisfactory. No development was obtained in dried beef or dried herring in which the fat may have been in a physical state that prevented feeding. Other fatty foods such as dried milk and dried egg were poor due almost entirely to a deficiency

of vitamins which can be remedied by addition of yeast. Commercial whalemeal (fat content about 16%) compared poorly with a substance like grassmeal (Table XVII).

Fraenkel and Blewett (1943c,d) have also shown that *P. tectus* requires accessory food substances, in particular a sterol (cholesterol, sitosterol or ergosterol) and seven factors of the vitamin B complex.

Substances with perfectly satisfactory food values may be unsuitable for physical reasons, such as hardness. These and other foods may be improved if they contain an abundance of easily used fine particles.

Population density.

At 70 per cent. R.H., experiments were performed at 23° and 20°C. to determine the effect of population density in small containers. At 23°C., densities of 1, 2, 4 and 8 newly-hatched larvae per $2 \times \frac{1}{2}$ inch tube were used and at 20°C. densities of 1, 4, 8 and 16 larvae per tube. Moisture-conditioned wheatfeed was measured by volume into each tube, the weight being 160 to 180 mg. per tube. Thus the minimum amount of food provided per larva was 10 mg., sufficient in itself to ensure that mortality or retarded development would not be due to food shortage (Gunn & Knight, 1945). Only at the density level of 16 per tube would food shortage be expected to reduce the adult weight.

TABLE XXII.

Mortality of *P. tectus* bred under different degrees of crowding at 70% R.H.

Temp. °C.	Original number of larvae per tube	Number of larvae per tube spinning cocoons	Number of tubes	Total number of larvae used	Mortality % of original number	
					Before cocoon formed	After cocoon formed
20	1	1	40	40	17	2
"	4	3 or 4	16	64	6	3
"	8	5 to 8	14	112	10	3
"	16	3 to 13	15	240	42	23
"	"	3	1	16	81	6
"	"	6	1	16	62	12
"	"	7	2	32	56	28
"	"	8	2	32	50	22
"	"	9	1	16	44	19
"	"	11	5	80	31	31
"	"	12	2	32	25	28
"	"	13	1	16	19	0
23	1	1	40	40	10	0
"	2	1 or 2	20	40	2	0
"	4	2 to 4	20	80	10	0
"	8	4 to 8	10	80	16	5

Larval mortality was markedly increased only at a density of 16 larvae per tube (Table XXII), but was unusually heavy with only one larva per tube. In addition, at the highest density large larvae frequently entered cocoons made by other quicker growing larvae and killed the larva or pupa inside. In this way one or two large larvae killed as many as 8 larvae and pupae in a single tube. Insects in cocoons were killed by other larvae in 13 of the 15 tubes which originally contained 16 larvae. Fig. 13 shows that, at this particular density, in tubes in which larval mortality was highest, growth was slowest. The insects spinning the first cocoons were especially vulnerable to the attack of feeding

larvae, but only 11 of the 56 insects killed in cocoons died before pupating, and not more than one of these was in any one tube. Therefore the developmental period of most of the individuals dying in cocoons is included in the results figured, and the accidental cannibalism of quick growing larvae inside cocoons is only slightly responsible for increasing the observed mean developmental period of larvae.

It is likely that those larvae which feed on other insects in their tubes gain considerably in weight. In seven of the 13 tubes mentioned above as being affected by cannibalism, one beetle was markedly heavier than the rest in each tube, and in six of these the heavy beetle was the last to pupate and to emerge, and is therefore assumed to be the individual responsible for the death of some at least of the other insects.

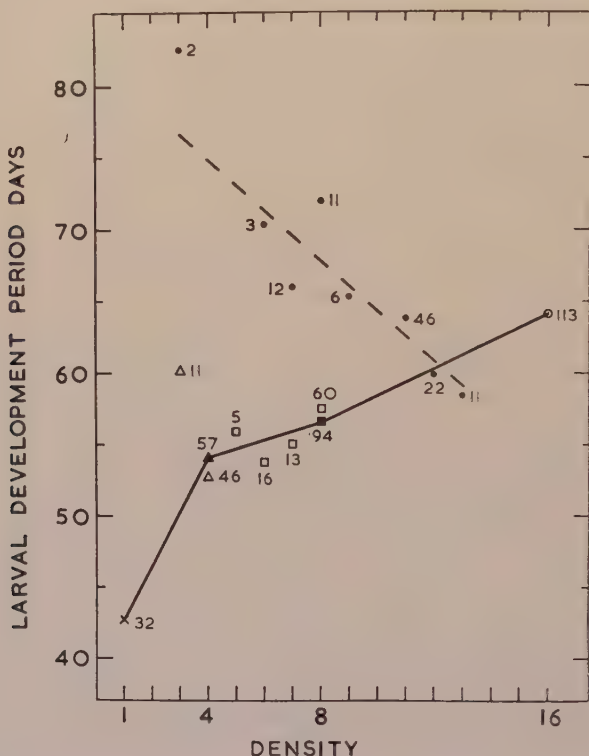


Fig. 13.—Effect of the number of larvae bred in $2 \times \frac{1}{2}$ inch tubes of wheatfeed on mean larval developmental period at 20°C. and 70 per cent. R.H. The full line represents the effect of original larval densities of 1, 4, 8 and 16.

The broken line represents the effect on mean larval developmental period of the number of larvae forming cocoons (cocoon density) at the original larval density of 16 ($b = -1.75$, $P < 0.001$).

Original density 16 = ○ and cocoon densities resulting •

8 = ■ 4 = ▲ 1 = x

The numbers given against the points are the numbers of larvae on which the mean larval periods are based.

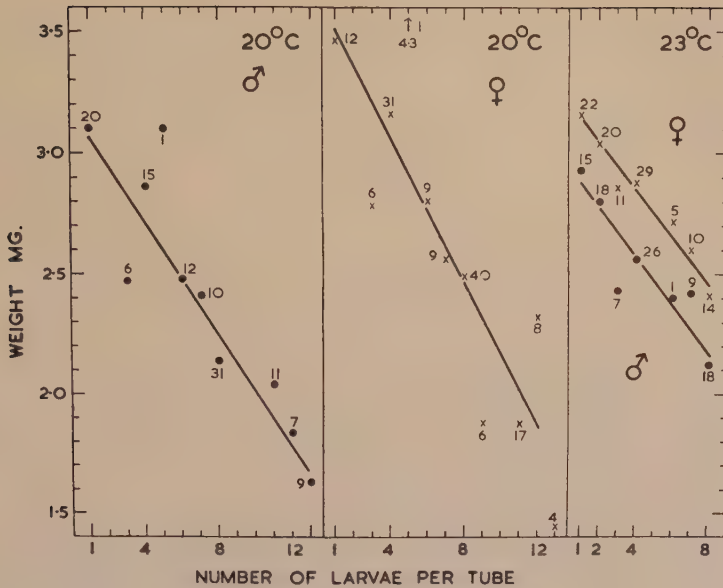


Fig. 14.—Mean weight of *P. tectus* adults bred at 70 per cent. R.H. at various degrees of crowding in $2 \times \frac{1}{2}$ inch tubes containing wheatfeed at 20° and 23°C. The densities plotted are the number of larvae building cocoons.

The numbers given are the numbers of larvae on which the means are based. All the regression lines are highly significant, the slopes being:— 20°, ♂, $b = -0.12$; 20°, ♀, $b = -0.15$; 23°, ♂, $b = -0.10$; 23°, ♀, $b = -0.10$.

TABLE XXIII.

Effect of crowding on developmental period at 23°C. and 70% R.H.

Original number of larvae per tube	Larval developmental period in days			
	N	M	±	S.E.
1	36	33.5	± 0.2	
2	39	38.1	± 0.4	
4	70	43.2	± 0.6	
8	62	44.6	± 0.4	

The developmental period of larvae was increased by increasing density. Even at the low densities of 2 or 4 larvae per tube the larval period was much greater than at a density of one larva per tube (20°C., fig. 13; 23°C., Table XXIII). Thus here the same "group effect" as that recorded by Gunn and Knight (1945) is apparent. They used much more food per insect and found that a large difference in larval developmental period occurred between a density of one and densities of two or more even though (in their experiments) the weight of food and the amount of space for each larva was *unaltered*. The weight of adult beetles was decreased by increasing density (fig. 14). The weight difference between the density of one and densities of 2 and 4 was comparatively less marked than the difference between the larval periods at these densities, indicating a much smaller group effect on weight than on larval period. The results of Gunn and Knight (1945) show a similar relationship. The increase in larval period and decrease in weight may be partly due to disturbed feeding, and, at 16 larvae per tube, to shortage of food.

High density decreased the pre-emergence period (23°C. , $b = -0.30$, $P < 0.001$; 20°C. , $b = -0.21$, $P < 0.001$). More handling is necessary when tubes contain many insects and disturbance by emerged insects before they are removed from the tubes is greater at higher densities. This disturbance reduces the resting period of the mature beetles in their cocoons, and this was probably the only cause of the decrease in the pre-emergence period.

Density did not affect the sex ratio.

Adult Life and Egg Output.

Hinton (1941) gives a detailed description of the adult beetle of *P. tectus* and includes it in a key to Ptinid beetles found in stored products. In the adult there are no external differences between the sexes. In this work individuals were sexed for experiment either as pupae, or as adults by gently pressing the abdomen of the newly emerged beetle, thereby causing the genitalia to protrude. A total of 6,755 individuals were sexed, of which 3,399 (50.3 per cent.) were females, indicating a 1:1 sex ratio. Hickin (1942a), however, found that only 46.8 per cent. of 1,493 beetles were females which is significantly below a 1:1 sex ratio ($P < 0.05$, using a chi squared test).

The average weight of female beetles at emergence from the cocoon is higher than that of males. This was also noted by Gunn and Knight (1945).

The behaviour of the adult beetle has been studied by a number of workers. Bentley (1944) found that the reaction to humidity depended on the state of desiccation of the beetles. Thirsty beetles collected at the wet end of a humidity gradient, but beetles that were not thirsty collected at the dry end. Bentley and others (1941) showed that in alternating light and darkness at 25°C. there was a diurnal rhythm of locomotory activity with maximum activity during darkness. This rhythm was continued for a few days in continuous light. Deal (1941), working on temperature preference, found that there was a preference for about 8°C. , the lowest temperature offered. This was not due to trapping at the cold end of the gradient as the insects were active at this low temperature, but could have been a positive reaction of thirsty beetles to high relative humidity. Gunn and Walshe (1942), who were able to avoid including a humidity gradient, found that in a constant temperature gradient beetles aggregated around two regions, 4° and 24°C. , and that aggregation around 4°C. was due to beetles being nearly immobilised at this temperature. They also found a cold avoidance reaction at about 10°C. in a gentle gradient but not in a steep one; and obtained movement away from regions at 30°C. or higher. Gunn and Hopf (1942) state that the amount of walking is not a simple function of temperature, but depends on culture temperature, on whether the temperature is constant or changing, and with changing temperature, on the speed and direction of change.

The adult beetle is negatively phototropic.

When disturbed, beetles readily adopt a death-feigning attitude; Hickin (1942a) described this attitude as being exactly that occupied by the pupa (Pl. IX, fig. 4). This is not quite accurate as adults feigning death hold their antennae ventral to the tucked in legs (Pl. X, fig. 1, i) whereas the antennae of the pupa are held dorsal to the first two pairs of legs and ventral to the third pair which are covered by the elytra and wings. This "pupal" attitude is adopted only when beetles fall on their backs and even then they frequently stop moving with the antennae extended laterally and the legs only partially tucked in. Beetles adopting the "pupal" position usually take longer to resume activity than those adopting any other motionless attitude. When beetles walking upright on a horizontal surface are shocked they do not assume the "pupal" position described by Hickin (1942a), but merely squat down, tucking the legs in slightly and holding the antennae laterally (Pl. X, fig. 1, ii), or they may stop immediately without altering at all the position of their appendages (Pl. X, fig. 1, iii).

Free liquid.

Several authors have emphasised the need of the adult beetles for free drinking water. Thirsty beetles adopt a typical attitude when drinking from a wet surface. They squat down close to the substratum with the legs tucked in close to the body and the antennae directed upwards and backwards. Plate X shows beetles drinking (fig. 2) and others running on blotting paper (fig. 3).

TABLE XXIV.

The length of life and the egg output of pairs of adults on fishmeal and of groups of adults in culture on wheatfeed, both provided regularly with drinking water.

Pairs on fishmeal.

Temp. °C.	R.H. %	Adult life of ♀♀ (days)		Adult life of ♂♂ (days)		Egg output in first 10 weeks		Total egg output	
		N	M ± S.E.	N	M ± S.E.	N	M ± S.E.	N	M ± S.E.
25	70	17	100 ± 9	17	91 ± 9	18	296 ± 27	17	373 ± 42
23	70	10	140 ± 12	9	137 ± 14	11	274 ± 19	10	516 ± 71
20	70	—	—	—	—	10	148 ± 18	—	—
15	70	—	—	—	—	4	103 ± 15	—	—

Cultures on wheatfeed.

Temp. °C.	R.H. %	Adult life of ♀♀ (days)		Adult life of ♂♂ (days)		Egg output in first 10 weeks Eggs per ♀	Total egg output Eggs per ♀
		M	Max.	M	Max.		
30	uncontrolled	69	124	78	115	60	63
27	70	168	276	172	311	159	261
25	70	185	360	181	319	134	276
20	uncontrolled	234	430	217	434	119	420
13	uncontrolled	387	515	383	476	16	76

TABLE XXV.

The length of adult life on wheatfeed of groups of *P. tectus* denied water.

Temp. °C.	% R.H.	Number of beetles	Adult life at the experimental conditions		
			M days	Min. days	Max. days
35	70	40	5.9	5	8
33	70	40	13.5	4	16
30	20	40	14.8	12	18
„	40	40	18.1	9	26
„	70	41	27.1	22	35
25	„	40	44.3	30	59
20	20	40	32.0	21	44
10	„	40	80.8	40	112
„	70	39	174.0	63	379
5	„	40	244.0	63	367

When placed at the experimental conditions the beetles had emerged from their cocoons for one to two days.

Similar data from Ewer and Ewer (1942).

Temp. °C.	% R.H.	Number of beetles	Median weeks	Min. weeks	Max. weeks
27	50	17	4—5	3	7
"	70	38	6—7	4	8
"	"	52	6—7	5	9
20	"	91	10—11	5	18
15	50	30	12—13	4	20
"	70	69	16	10	36
"	90	58	25	14	?

In the present work, a regular supply of drinking water has proved essential for the length of life and egg output to reach a maximum. This is shown by experiments described in later sections and is illustrated by fig. 17 and Tables XXIV and XXV. Braune (1948) obtained good cultures only when a regular supply of milk was given in addition to the normal diet of air-dried flies. With liquid milk alone egg output was good, the female used laying 491 eggs in 675 days and the male living for 1,136 days. On a diet of water alone only 150 eggs per female were laid. He also found that after a drink of milk, beetles ate dried flies more rapidly. Ewer and Ewer (1942) starved beetles for one week, then supplied food. The presence of drinking water increased the percentage of beetles that ate the food. They also found that drinking water was necessary for continued egg laying and Howe (1950a) made a similar observation for beetles in a warehouse.

Experimental methods.

Experiments to measure egg output and length of adult life were of two types, one using isolated pairs and the other using groups of 50 beetles.

Each isolated pair was kept in a 2×1 inch glass tube on sieved fishmeal. A cork with a hole bored in the centre and covered with cotton cambric prevented the escape of beetles which sometimes climbed the glass. Eggs were removed with a sieve of 60 meshes to the inch which retained the eggs but allowed the food to pass. At each sieving the beetles were freed from food with a small soft brush, placed on moist cotton wool, and allowed to drink for two hours or longer. Excess water must be squeezed from the cotton wool, because if the beetles are wetted food sticks to them when they are returned to the laying tubes. Drinks were given daily except on Sundays at 25° and 23°C., twice weekly at 20°C. and weekly at 15°C. In our experience, to obtain the maximum egg production, the interval between drinks should not be more than three days at 23°C. or above and not more than one week at 20°C. or below. The food was renewed at intervals as necessary. An increase in the daily number of eggs laid was frequently observed on the day following the addition of fresh food, but the number reverted to normal within three or four days.

The technique was similar for experiments with groups of beetles. Groups of 50 beetles were used and kept on sieved wheatfeed about $\frac{1}{4}$ -inch deep in 1 lb. jam jars. After removal from the food with a coarse sieve, the beetles were allowed to drink on moist blotting paper. Drinks were given daily except on Sundays for the first month, then every two days, except at 13°C. where drinks were given every two days from the start of the experiment. At each temperature four groups of 50 beetles were used and as far as possible, the density in each jar was kept at between 40 and 60 beetles. As soon as the average number in all jars fell to about 40, the beetles from one jar were shared between the others. In the last jar the density was allowed to fall to zero, concluding the experiment.

Length of adult life.

In general, with both pairs and groups of beetles for which drinking water was provided, the average lengths of adult life of males and females are about equal

(Table XXIV). Using beetles given no drinking water, Braune (1948) found that males lived longer than females, and Ewer and Ewer (1942) record an indication that males lived slightly longer than females.

Increase in temperature shortens adult life. This is shown by Table XXIV for experiments at temperatures between 13° and 30°C. with beetles given drinks, and by Table XXV for experiments between 5° and 35°C. with beetles denied drinking water. At 5° and 10°C. condensation was observed on the glass tubes during the examinations at room temperatures made twice weekly for these two conditions. It is possible that beetles were able to drink this condensation, and so lengthen their adult life. Comparison of Tables XXIV and XXV shows that adult life is much longer when drinking water is provided. Additional evidence for this was obtained at 13°C. and uncontrolled humidity. At this condition, without drinking water, female beetles lived a mean of 50 days (maximum 75 days) and male beetles a mean of 53 (maximum 95). These periods are much shorter than those shown in Table XXIV for beetles which were provided with drinks. At 23°C., in the experiment illustrated by fig. 17, most of the beetles denied drinks were dead in 36 days but only a few of those given drinks were dead by this time.

Eight beetles all survived exposure for 58 days in an experiment at low temperature varying in the range $-0.4^{\circ}\pm 2.3^{\circ}\text{C.}$, the relative humidity being approximately 70 per cent. In a similar experiment in the range $-2.8^{\circ}\pm 2.1^{\circ}\text{C.}$ all of the eight beetles exposed survived for 36 days, but none of another set of eight survived 56 days' exposure. Thirteen out of twenty survived exposure to $-10.8^{\circ}\pm 1.4^{\circ}\text{C.}$ for 3 days. Hickin (1942a) found that adults survived and laid eggs after exposure for one week to outside conditions with the temperature varying between -8° and 2°C.

As would be expected from these results, *P. tectus* normally survives the winter in warehouses in Britain. Miss B. E. Adamson of this laboratory exposed cultures to outdoor conditions for the winter of 1950-51 in a Stevenson screen in which the minimum temperature recorded was -5°C. using a recording thermograph accurate to 1°C. Insects in these cultures survived the winter and bred in the following summer. Mansbridge (1936) found that both larvae and adults in cultures survived the winter of 1935-36 in an unheated building in which the minimum temperature was -2°C. (accurate to $\pm 1^{\circ}\text{C.}$).

In the presence of food and with drinks provided, humidity has little effect on the duration of adult life, but if water is withheld, life is shorter in a low humidity (Table XXV). The results of Ewer and Ewer (1942), also shown in this Table, fit in well with the present work. In the experiments at 23°C. illustrated by fig. 17 adults not given drinks died more quickly at 50 per cent. R.H. than at the higher humidities.

Thus high temperature, absence of drinking water and low humidity all reduce the length of adult life. In the warehouse these factors usually occur together and each reinforces the effect of the other.

Egg production.

At 23° and 25°C. and 70 per cent. R.H., laying of fertile eggs commences two or more days after emergence from the cocoon. The shortest time obtained by Braune (1948) between mating and production of the first egg was two days at an unstated temperature. Ewer and Ewer (1942) state that at 25°C. and 70 per cent. R.H., ripe eggs were present in the ovaries at three to twelve days after emergence; that testes and sperm sacs of males contained active sperm at emergence and that males could copulate within 24 hours of emergence. Braune (1948) confirmed these conclusions and also found that the upper part of the testicular ducts contained immature sperm a month or two after emergence.

Braune states that the first ova are formed in the ovaries during the pre-emergence period and may be fertilised immediately upon emergence of the female, which requires no food before mating. He also found that the sperm from one copulation lasted for 145 days at a varying temperature so the female requires to copulate several times in her span of life.

In general, fewer eggs per female were laid by females in groups than by the females in isolated pairs. This may be due partly to crowding. The only temperature-humidity combination used both for pairs and cultures was 25°C. and 70 per cent. R.H., but here fishmeal was used as food for pairs and wheat-feed as the food for groups, hence food probably influenced the difference observed. This contention is supported by the results of a later experiment in which, over a period of a week, egg production was poorer on wheatfeed than on fishmeal. The total egg output per female was greatest at 20° to 23°C. The largest number of eggs from a single female was 962, recorded at 23°C.

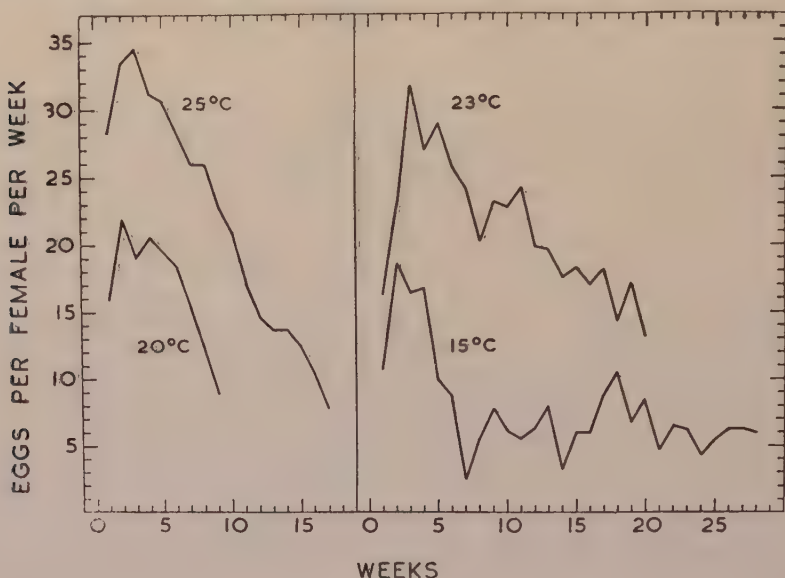


Fig. 15.—Egg production per initial female at 70 per cent. R.H. on fishmeal in experiments with pairs of beetles. The curves are based on 4 pairs at 15°C., 20 pairs at 20°C., 15 pairs at 23°C., and 19 pairs at 25°C.

Figs. 15 and 16 show the effect of temperature on the rate of egg output. During early adult life the rate of egg output was greatest at 27°C. (see also Table XXIV). Later in adult life, however, the rate at both 27° and 25°C. fell well below that at 20°C. (fig. 16).

Gunn and Knight (1945) kept seven pairs of adults at 24.7°C. until one member of the pair died. These laid 501 to 803 eggs each (mean 647) and one female was remated after the death of her mate and laid 960 eggs in all. During the first three weeks, six pairs laid 17, 41 and 37 eggs per female per week. This is about the same as the initial rate per female shown in fig. 16, but the average total number of eggs laid is much greater than that shown in Table XXIV.

Ewer and Ewer (1942), in experiments with groups of beetles at 25°C., obtained ten eggs per female of unstated age per week for three weeks. This is similar to the results given in fig. 16 for beetles at the age of 5 to 15 weeks.

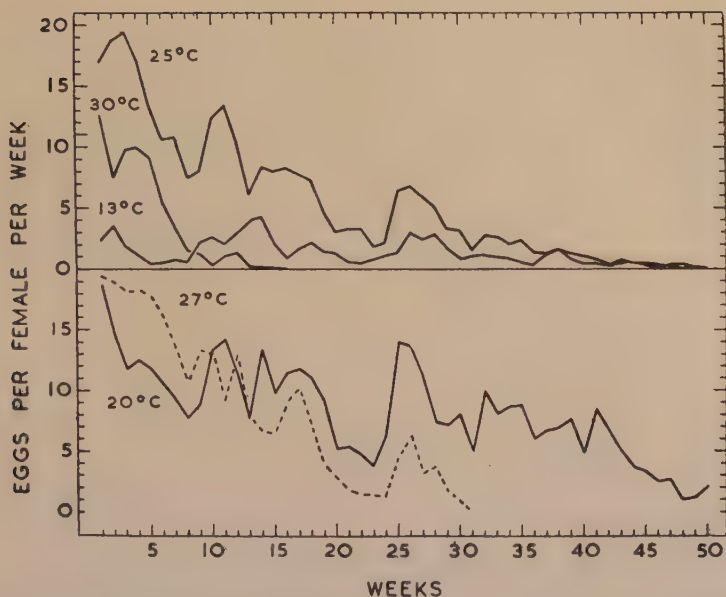


Fig. 16.—Egg production per initial female in experiments using groups of beetles on wheatfeed at 70 per cent. R.H. for 25° and 27°C., and uncontrolled humidity for 13°, 20° and 30°C.

The curves are based on 200 beetles at each temperature.

In addition, Ewer and Ewer found that a total of about 25 eggs per female was laid at 30°C. (using 19 beetles of unknown sex and assuming a sex ratio of unity). They also found that oviposition almost ceased after 13 days and that gonads were abnormal after exposure to 30°C. for several hours. This egg number is smaller than that given in Table XXIV. The maximum temperature for oviposition is probably little above 30°C.

Ewer and Ewer (1942) estimate the lower limit for oviposition as 5° to 7°C. and fig. 16 shows there is a continuous but slow egg output at 13°C. Warehouse observations (Howe, 1950a) indicate that oviposition may occur below 5°C.

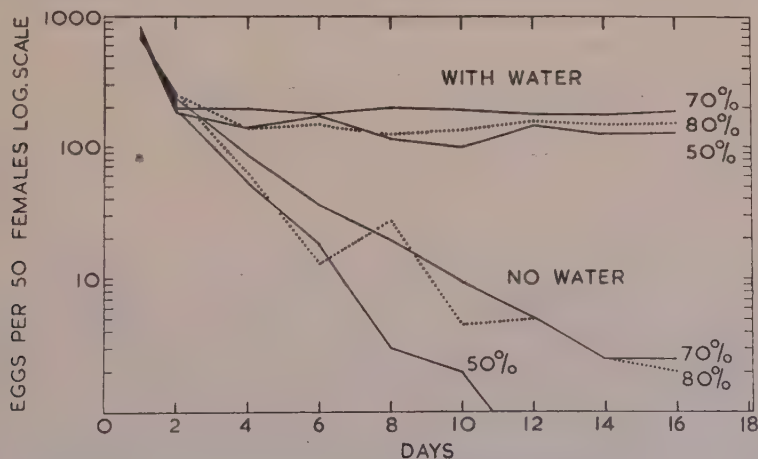


Fig. 17.—The effect of relative humidity and drinking water on the egg production of groups of *P. tectus* at 23°C.

An experiment at 23°C. to test the effect of humidity on egg production is illustrated by fig. 17. Young adults were given ample drinking water at emergence from the cocoon and kept until enough had emerged to divide them into six lots of 100 beetles. Two lots were placed at each of three humidities, on humidity-conditioned fishmeal passed through a sieve with 60 meshes to the inch. The food was sieved for eggs, first daily, then every two days. One lot at each humidity was given a drink after each sieving, the other lot being denied drinks. With water provided, humidities between 50 and 80 per cent. R.H. had no effect on egg output, but with no water, egg output diminished more quickly at 50 per cent. than at 70 or 80 per cent. R.H.

Choice of site and food for oviposition.

When food is available beetles oviposit in it. Newly laid eggs are sticky and when the layer of food in a tube is very thin, they adhere to the glass under the food particles, some of which stick to the egg shell. Ewer and Ewer (1942) state that eggs are laid readily in knitting wool in the absence of food. In our experience, however, eggs were laid only occasionally in thick brown knitting wool in the presence of food and only slightly more frequently when food was absent. Larvae cannot develop on clean wool. Braune (1948) found that eggs were commonly laid in cotton wool which had dried out after being soaked in milk, and also in the corners of his milk troughs. When cracks and crannies are available, eggs are commonly laid in them when food is present. Braune (1948) provided dead flies as food and found that eggs were usually laid in the crevices on the body. With bagged commodities, eggs are often laid through the meshes of the sacking material into the food.

In unfavourable conditions oviposition stops or is reduced. Thus, when beetles are kept in glass containers without food, no eggs are laid until a layer of frass has accumulated and then only a few are laid. When fresh food was provided in oviposition experiments more eggs were laid for the next few days. Braune (1948) considers that the abdomen is not large enough to hold as many as ten mature ova, but Mr. D. W. Hall of this laboratory found as many as 14 mature ova in one female. The usual number found in females from healthy cultures was about eight with fewer in females from poor cultures. Mr. Hall also found mature eggs in the process of resorption. Thus in unfavourable laying conditions females probably break down unlaid mature eggs and store a small number of mature or nearly mature ova. It is also possible that they can delay the maturation of ova, a return to favourable conditions restoring the normal rate of maturation.

Beetles given a choice of four foods, fishmeal, wheatfeed, flour and copra in which to oviposit showed no decided consistent preference. In three replicate experiments there was a slight indication (not statistically significant) that fishmeal and wheatfeed were preferred and copra disliked (see Howe & Burgess, 1952, fig. 6).

Short term effect of the type of food on egg production.

The effect of food on egg production was determined over periods of one week at 20°C. and 70 per cent. R.H. using fishmeal, flour, wheatfeed and ground copra cake on all of which adequate larval development is possible. Four groups of 100 young adults were selected at random from cultures containing fishmeal plus yeast as food. The experimental samples of food were each ground and passed through a sieve of 60 meshes to the inch so that eggs could be removed later by sieving. Each group of beetles was put at the experimental conditions for 5½ days on an unsieved sample of the appropriate food to condition them to that food. After this the group was placed on moist blotting paper for half a

day and then placed for 24 hours on the experimental sieved sample of the food to which they had been conditioned. This process was repeated putting each group of beetles on each of the different foods in turn. Thus, over four weeks each group spent one week on each food and the numbers of eggs laid on the last day of the week counted. Each group of beetles was passed from one food to another in a different order, so that for example, one group started on wheatfeed and the remaining three groups were transferred, to wheatfeed, one from each of the other three foods. The containers used were 1 lb. jam jars in a desiccator. All food was conditioned to humidity before use.

The results were examined by an analysis of variance technique and are given in Table XXVI. Large differences occurred between the foods, far more eggs being laid on fishmeal and copra than on wheatfeed or flour. Differences in the number of eggs laid in the different foods were all statistically significant except the difference between fishmeal and copra.

TABLE XXVI.

The short-term effect of four foods on the rate of oviposition.

Food	Eggs laid by the 4 groups of adults	Total	Source of variation	F	P
Fishmeal	211, 255, 397, 242	1105	Between foods	16.5	<0.001
Copra ..	178, 298, 220, 332	1028	Between groups	5.0	0.05-0.01
Flour ..	104, 224, 176, 220	724	Between days	4.8	0.05-0.01
Wheatfeed	99, 105, 140, 126	470			

These results show that the food in which the largest number of eggs are laid is not necessarily that food on which larval development is quickest. Also, when given a choice of foodstuffs in which to oviposit, adults of this species showed no marked preference either for the foods on which their egg output was highest (Table XXVI) or for those on which development was quickest. For instance, in the experiment on choice of foods, rather more eggs were laid on fishmeal and wheatfeed than on copra or flour, but as Table XXVI shows, egg production was good on copra as well as on fishmeal and was poor on wheatfeed as well as on flour. Although egg output of beetles confined on fishmeal was high and there was a tendency for this food to attract oviposition, larval development on it is not so rapid as on wheatfeed (Table XVII).

Howe and Burges (1952) obtained a more striking result with *Eurostus hilleri* Reitt. using the same four foods. This species laid more eggs when confined on fishmeal than on any of the other three foods, but it could not complete its development on fishmeal. However, when a choice of foods was given most eggs were laid on wheatfeed and flour and only a few on fishmeal.

Genetics.

Two easily visible morphological characters of adult beetles were noted which may be inherited. The first was the size of the hind wings described by König (1936), Waddington (1942) and Braune (1948) as atrophied and macropterous forms. The atrophied wing is the usual form, being found in over 90 per cent. of the thousands of individuals examined. Several intermediate stages exist between the usual reduced form (subsequently called "short") and the full coleopterous wing (subsequently called "long"), two being fairly distinct but comparatively rare. Wing reduction may occur unequally, one wing being long and the other short. Waddington states that the reduction of the wings takes place exclusively during the pupal period and is caused primarily by necrosis of cells throughout the whole extent of the wing epithelia.

Long-winged parents tended to have a greater proportion of long-winged offspring than did short-winged parents. Thus many pairs in which both parents were short-winged produced no long-winged offspring. One pair produced 421 live offspring including only 4 with long wings, all appearing in a short period from eggs laid in the middle of the life of the female parent. In the next generation all the 190 beetles examined were short-winged. Another pair of short-winged parents produced 127 short-winged beetles. When the female died, the male was crossed with a long-winged female which produced 27 long-winged beetles in 160 examined. The largest proportion of long-winged individuals noted, 27 out of 41, was from a pair of long-winged parents. Further information is given by a series of brother-sister matings in which 14 short-wing crosses gave 8 per cent. long-winged in 483 individuals; six long-wing-short-wing crosses gave 15 per cent. long-winged in 194 individuals; one long-wing cross gave 4 long-winged out of nine.

Long-winged beetles were significantly heavier than short-winged beetles in both sexes.

	Females					Males			
	N	M	± SE	P		N	M	± SE	P
Long-winged	143	3.75	± 0.03	< 0.001		95	3.51	± 0.05	< 0.001
Short-winged	589	3.50	± 0.02			712	3.21	± 0.02	

Braune considers this character to be atavistic, but the above evidence suggests that there may be some complex hereditary mechanism involved.

The second heritable character noted was an emargination of the tip of each elytron. Occurrence of this character was noted as follows in individuals of known parentage.

No. of parent pairs and type of cross	Offspring examined	Emarginate offspring
24, normal × normal	737	mean 4%, max. 5 out of 21
2, normal × emarginate	66	10 individuals
	13	1 individual
1, emarginate × emarginate	2	1 "

Discussion.

In view of the biology of *P. tectus* described in this paper, it is no surprise that it is a serious and widespread pest of warehouses in humid and temperate zones. Few foodstuffs cannot be attacked and there cannot be many warehouses in these areas which are sufficiently cold for long enough to kill the more resistant stages. High temperature is the chief limiting factor which restricts its distribution, but low temperature, low humidity and lack of free drinking water prevent its rapid increase. At its optimum, it breeds much more slowly than most other widespread and serious pests, but even so it is capable of doubling its numbers in a fortnight and it also continues to develop at much lower temperatures than most other pests.

Comparison of the results given in this paper with similar but less detailed work on other species of the family (Howe & Burgess, 1951, 1952) shows that the greater abundance of *P. tectus* can be reasonably explained by the more rapid development and the higher oviposition rate of this species. A more detailed comparison of the Ptinid species is made in another paper (Howe, 1953) by a method which converts all the biological data available at given conditions into a single figure which represents the potential rate of increase of the species at those conditions.

The food range of *P. tectus* is no less wide than that of any other Ptinid species. It has been commonly assumed (e.g., Braune, 1948) that *P. tectus* thrives best on foods of animal origin and in fact at this laboratory the beetle, for years, was bred on fishmeal. The present work shows that the species does better on many vegetable foods.

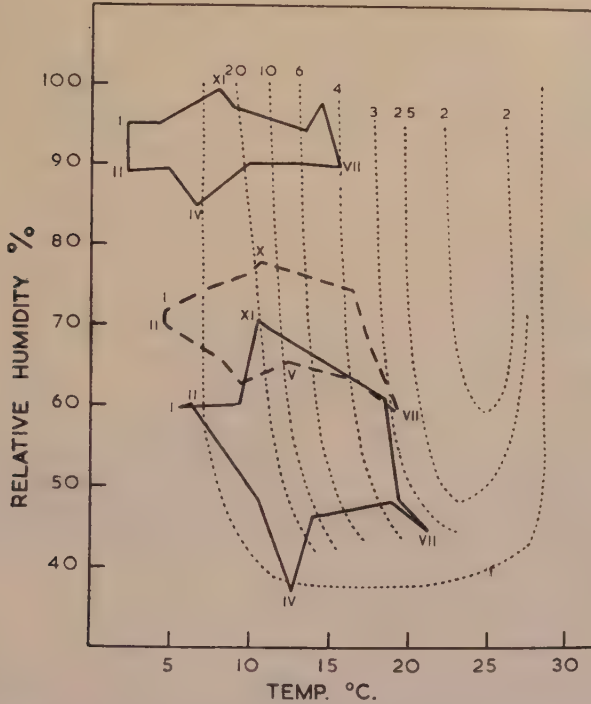


Fig. 18.—Diagram relating rate of increase of *P. tectus* to physical conditions recorded in a warehouse at Brentford during 1951. I = January.

The results given in this paper may be used to decide if *P. tectus* is likely to become a pest in any place by comparing fig. 11, or some similar diagram, with a climatograph of that place by superimposing one on the other as in figs. 18 and 20. The portions of the climatograph falling outside the limits are particularly important. For instance, for Kano in Nigeria the *mean* conditions are continuously too hot or too dry for the development of *P. tectus* in about eight months of the year. This reasoning must be used with caution because we are trying to predict on the basis of results obtained at constant conditions what might happen at variable conditions. In addition, it is the temperature and moisture content of the *produce* which are important, rather than the outdoor conditions which are given in meteorological records. In Kano the temperatures in stacked produce out of doors happen to be higher than the air temperature (Howe, 1952), but, except when produce is heating, warehouse temperatures are more equable than readings taken out of doors. Similarly, minimum temperatures during winter inside an unheated building at this laboratory have been of the order of 10°C. above the minima recorded in a Stevenson screen in the grounds.

Actual warehouse conditions from thermo-hygrograph records for a warehouse at Brentford, near Kew, are shown in fig. 18. In this figure, dotted lines connect points (representing combinations of temperature and humidity) for which equal

periods are required for a stable population of *P. tectus* to double itself, as calculated by the method of Birch (1948) from the results given in this paper. These periods are given in weeks. Heavy lines enclose the conditions of air temperature and humidity experienced in the warehouse. Three climatographs are given; for maximum humidity, minimum temperature (upper complete line); for minimum humidity, maximum temperature (lower complete line); and for dusk humidity and temperature (broken line). The points shown are the means for each month of the daily maximum, minimum, and twilight temperatures and relative humidities. The points for the months June to September all fall in the zone between the lines showing conditions in which a population of *P. tectus* is capable of doubling in three to six weeks. This agrees closely with the warehouse observations given in Howe (1950a) of the doubling of the population in just under six weeks throughout these months.

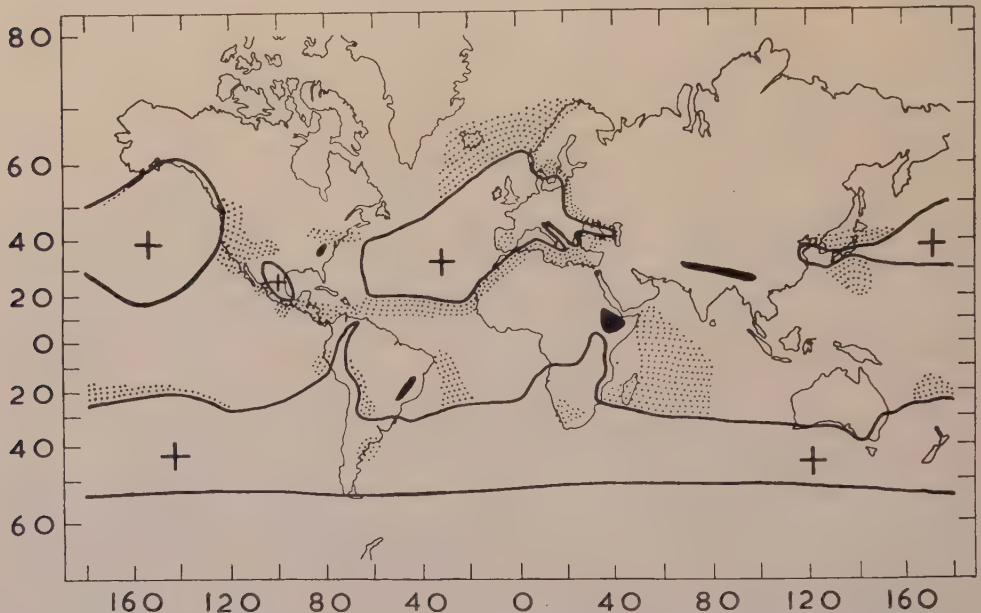


Fig. 19.—The theoretical range of *P. tectus*, marked + or shaded black, as estimated from laboratory data. Doubtful areas are stippled.

A slight modification of this approach has been used to construct a map (fig. 19) which is intended to show those areas in which *P. tectus* can maintain itself continuously.

Summarised standard climatic data for nearly 1,000 places outside the British Isles were studied. The mean daily maximum and minimum temperatures for each month were examined and the number of months with temperatures outside the limits -4.5° to 31.5°C . noted. For places with temperatures outside these limits, the points half way between the monthly maxima and minima were found and where these were outside the above limits for two or more months the place was considered totally unsuitable for *P. tectus*. The remaining places were considered favourable if the temperature ranges fell within these limits and doubtful if the ranges extended beyond these limits for two months or more.

After this preliminary sorting a number of further criteria were applied to both favourable and doubtful groups. If the mean monthly maximum (the mean of the highest temperature recorded in the month over a number of years)

exceeded $35^{\circ}\text{C}.$ for two months or more, the place was considered unfavourable since ten days continuously at $35^{\circ}\text{C}.$ is completely lethal (fig. 11). Similarly, nine months of the year or more exceeding $29.5^{\circ}\text{C}.$ were deemed unfavourable.

If the highest mean monthly maximum temperature reached $35^{\circ}\text{C}.$, the place was considered unfavourable if the mean *daily* minimum temperatures for the hottest two months did not fall to $21^{\circ}\text{C}.$ or doubtful if the mean minimum fell below $21^{\circ}\text{C}.$

This criterion is open to question because of lack of evidence on the effect of temperatures varying continuously between favourable and unfavourable levels. Stacks of produce normally show only comparatively slight diurnal variations probably about the midpoint of the daily range. Consequently, if the daily range is 35° to $21^{\circ}\text{C}.$ the produce temperature may be reckoned to be about $28^{\circ}\text{C}.$ so that feeding larvae at least will be exposed to continuously excessive temperatures.

For a few of the places already reckoned unfavourable because the mean monthly maximum exceeds $35^{\circ}\text{C}.$ for two months, if the mean daily minimum for these months fell to $15.5^{\circ}\text{C}.$ or below, they were transferred to the doubtful category.

No additional information could be gained by using further criteria for minimum temperatures or by considering relative humidities.

Places expected from this reasoning to be suitable to *P. tectus* are chiefly those with temperate or maritime climates. This is obviously modified by height, a number of mountainous areas in the tropics being suitable, the three largest of which are marked on the map. Conversely, some mountainous areas in Europe are too cold.

In the cold areas the insect can breed in heated premises. This accounts for records in Finland, Leningrad (Shapiro, 1941), Platinum in Alaska and east Canada (see p. 463).

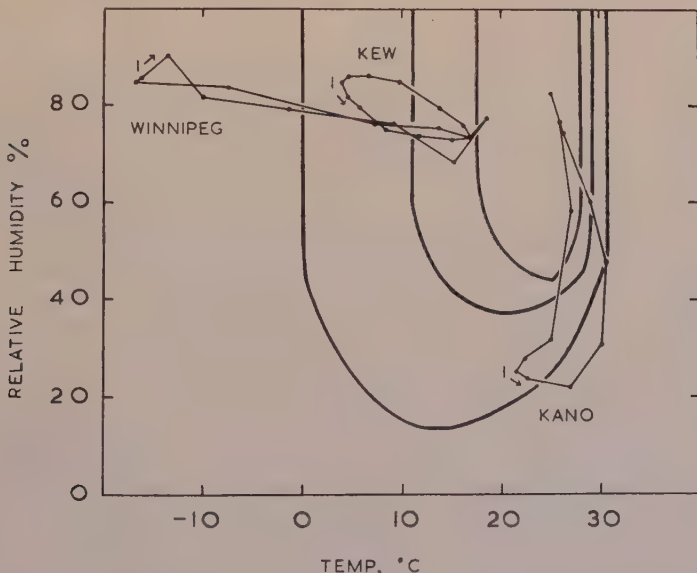


Fig. 20.—The relation of climate to the survival and development of *P. tectus*.

The relation of climate to survival and development is shown graphically in fig. 20. Climatographs of the mean daily temperature and humidity for each calendar month are given for Winnipeg in central Canada, Kew in England, and

Kano in Nigeria (the mean being the mean of the half-way points between the daily maxima and minima). Heavy lines enclose three zones, an inner zone of rapid development, then one of moderate development, then one of very slow or no development, beyond which lies the outer lethal zone. The inner heavy line joins conditions of temperature and relative humidity at which development is completed in 100 days on wheatfeed, the middle line those for development in 300 days, and the outer line joins conditions completely lethal in about 100 days. Winnipeg can be seen from fig. 20 to be definitely unfavourable for *P. tectus* being much too cold for six months of the year. Survival out of doors would be expected from May to October and possibly a month or two more in unheated buildings. Specimens of *P. tectus* were included in a sample of flour infested by *Ptinus villiger* Rtt. sent to us by the Grain Research Laboratory, of Winnipeg, but *P. tectus* is not recorded as a serious pest anywhere in Canada. Brown (1940) describes it as abundant in British Columbia, which is marked as a favourable area in fig. 19, and he also gives three records for eastern Canada.

Of the areas designated favourable in fig. 19, *P. tectus* appears to be abundant only in British Columbia, New Zealand, and north-west Europe. No doubt the continuously high relative humidity in these places has contributed to its increase. It is noteworthy that in all these areas no mean monthly temperature exceeds 20°C. The only other favourable areas in fig. 19 that meet this requirement are the high Andes and the southern tip of S. America from latitude 40°S.

There are few or no records in a large part of the area marked as favourable for the species, namely South America, southern Africa, south-eastern Australia, Japan, Mexico, southern Europe (see p. 463). This in part is because the insect has not been introduced. Chile, for example, is thought to be the original home of *Trigonogenius globulus* Sol., Japan of *Eurostus hilleri* (Rtt.), and *Stethomezium squamosum* Hinton occurs in South Africa. These three species are fairly close to *P. tectus* in climatic and food requirements and also are less prolific, hence *P. tectus* could certainly live in these places if introduced. On the other hand, spread of *P. tectus* from Tasmania, generally accepted as the original home of the species, to the mainland of Australia and also spread of the species southwards in Europe should not be geographically difficult, so that on balance these are probably marginal areas. A complicated picture was obtained for southern Europe from the analysis of climatic data. The zone designated doubtful because of heat included Greece, Adriatic Italy, central Iberia (Lagos to Bilbao), the islands from Sardinia to Crete and a few places in southern France. The Adriatic Balkan coast was considered favourable. The lower Danube was mainly favourable with some points doubtful because of heat and a few doubtful or unsuitable because of cold.

In Britain, *P. tectus* can be found at all seasons in all stages, though it is most numerous in September and October (Howe, 1950a). On the mainland of Europe it is less commonly recorded than *Ptinus fur* (L.) which it has largely superseded as a pest in Great Britain.

According to Zacher (1939 a, b), *P. tectus* reached Britain before any other European country, but it is not clear whence it came. This species is seldom found on imports into Britain (Freeman, 1948). It is unlikely that many individuals could travel successfully in bulk grain, but any kind of bagged produce would provide a suitable and fairly safe habitat for travel. Heavy infestations should be made obvious by the cocoons, but a slight infestation with cocoons only found on the inside of sacks could sometimes escape the notice of quarantine inspectors. Originally, the journey across the tropics may have been a barrier to spread, but speedy transport made this possible. The most resistant stages of *P. tectus* can withstand 30° to 32°C. for 50 to 100 days. If hold temperatures remain in this region, a fairly reasonable tropical shade temperature, this species should be able to travel across the equator with little difficulty on normal trade

journeys. Probably the chief reason why *P. tectus* is rarely found on ships is that the majority of British imports are from unsuitable climatic areas or countries free from this pest. Of the areas from which *P. tectus* is recorded the only one which supplies Britain with a large quantity of imports is Canada, and from there most of the imported produce is grain in bulk. These views receive support from the fact that *P. tectus* has been recorded five times during the past year (1951-52) on ships carrying barley, peas and livermeal in bags from New Zealand. Over half the records of *P. tectus* on ship cargoes have been from Canadian flour.

The ability of *P. tectus* to live in warehouse dust and débris and its habit of pupating in warehouse fabrics has given rise to populations endemic to warehouses. Even in a modern brick and concrete warehouse at Brentford, where little débris and few spaces in the fabric existed, small numbers of *P. tectus* were common on many products, apparently picked up from the warehouse. Mr. C. W. Coombs, of this laboratory, found large populations of Ptinid beetles feeding on accumulations of weevil damaged grain débris in the cavities of the double walls of a warehouse. The biggest problem in the control of *P. tectus* is the elimination of such endemic populations, which necessitates not only the removal of obvious refuse but also improvement in warehouse design to eliminate cavities where accumulations of food and sweepings may collect.

Summary.

Ptinus tectus occurs on a large range of foodstuffs in all types of storage places, in warehouse refuse and in the nests of birds. It is widely distributed in cool and temperate areas but is very rarely found in hot climates. In Britain it can survive the winter when exposed in a Stevenson screen to outdoor shade conditions. *P. tectus* is seldom found on imported cargoes inspected aboard ship, but many British warehouses carry a population of the species resident in the fabric of the premises.

The larva is able to bore through cellophane, card and textiles and can also make impressions on wood. The fully fed larva spins a cocoon on the fabric of containers and buildings. Holing of packages and contamination by silk of high grade produce form the chief economic losses caused by the species, but occasionally large populations are found, sufficient to reduce the food value of produce. Unlike the larvae, adult beetles cannot penetrate sound linen bags, but lay eggs through the meshes.

In a series of consecutive experiments, performed identically as far as possible, variations in the length of developmental period and in emergence weight of adult beetles were greater than would be expected by chance. The variations could not be related to any single observed or suspected inconsistency of technique or environment. It is desirable therefore to conduct comparative experiments simultaneously.

Development is quickest between 23° and 27°C. The highest temperature at which the developmental cycle can be completed is 28°C., and the lowest temperature is between 5° and 10°C. Adult beetles are more resistant to fatally high temperatures than pupae or large larvae, which themselves are more resistant than eggs or young larvae. Adults were killed in 4 hours at 41°C. and large larvae at 38°C. At slightly lower temperatures which kill a little more slowly, adults (denied drinking water), pupae and large larvae had a similar resistance, all the insects used being killed at 35° and 33°C. in 8 and 16, 12 and 16, and 9 and 22 days respectively. At 30°C., when given drinking water, adults were again the most resistant stage and lived a maximum of 124 days. The relative resistance of the different stages is similar at very low temperatures, the adults being most resistant. At approximately -3°C. adults were killed by

an exposure of 56 days and large larvae by 13 days; they survived exposures of 36 and 6 days respectively. Transfer of larvae from near-optimal temperatures to temperatures at which development is slow and *vice versa* has only a transitory effect on the speed of development, which rapidly becomes stabilised at the normal speed for the new temperature. Beetles bred at low temperatures are heavier than those bred at high temperatures within the range in which mortality is low.

High humidity is favourable for development except when mould growth is very extensive. Development is quickest at 70–90 per cent. R.H. The lowest humidity which allows complete development is about 40 per cent. R.H. Development is slow at humidities below 50 per cent. R.H. and egg hatching is greatly retarded, probably as a result of hardening of the egg shell. Low humidities, however, sometimes slightly decreased the pre-emergence period. In favourable conditions, 80 per cent. or more eggs hatch and post-embryonic mortality is slight.

P. tectus has been bred on various natural and dehydrated foods. Development was rapid on wheatfeed, flours and other foods with a high carbohydrate content and also on fishmeal and some other foods with a high protein content. Development was not completed on greasy foods and mortality was very heavy on some foods which became sticky in moist conditions due to a high sugar content. Very hard foods, such as dried strips of potato were resistant to penetration by young larvae. Growth was usually more rapid if the foods were finely ground than if presented to the young larvae as lumps. The addition of five per cent. by weight of yeast improved most foods. Development occurred in some warehouse dusts and rat droppings. Larvae developed more quickly on a mixture of fishmeal and wheatfeed than on either food alone, indicating that each food has some deficiency which is made up by the other.

Experiments with synthetic foods showed that deficiency of sterol and absence of carbohydrate retarded development. The proportion of glucose to casein had much less effect.

When larvae were bred more than one to a tube, some cannibalism resulted. The developmental period increased progressively and the weight of beetles decreased as the number of insects per tube was raised, even though enough food per insect was present for normal development.

The sex ratio is approximately unity. The mean weight of female beetles is greater than that of males. A regular supply of drinking water is necessary for the maximum length of life and egg output. When water is supplied the length of life of female and male beetles is about equal. Adult life was longest at 13°C., females living for a mean of 387 days; it was shortened by increasing temperature until at 30°C. they lived for 69 days. Only when no drinking water is provided does low humidity shorten adult life.

At 23°C., oviposition commences two days after emergence from the cocoon. The female is capable of continuous egg laying over a prolonged adult life. Pairs of beetles kept separately lay more eggs than beetles kept in groups. The total egg output was greatest at 23 to 25°C. (mean 516 eggs) and the largest number of eggs recorded from a single female was 962. At 25°C., and above, the rate of oviposition was highest during the first few weeks. Then it steadily decreased, the rate of decrease being most rapid at the highest temperature. The greatest sustained oviposition rate exceeding 15 eggs per female per week for six weeks was at 27°C., which is near the maximum temperature at which development can be completed. At 27°C., oviposition ceased in about 30 weeks whereas at 20°C. the rate of egg laying declined less rapidly and averaged 5 eggs per female per week for more than 45 weeks. The highest temperature for oviposition is probably a little above 30°C. Oviposition is slow at 13°C., but can still take place below 5°C.

Eggs are normally laid in food and few are deposited in such materials as wool. In unfavourable conditions, *e.g.*, when food is lacking, mature eggs are

resorbed but a few are stored in the abdomen and are probably laid when favourable conditions return. Beetles confined on different foods for periods of one week laid more eggs in fishmeal and in copra than in wheatfeed or in flour. The food in which the largest number of eggs was laid was thus not wheatfeed, the food on which development is quickest. When given a choice of foodstuffs for oviposition, *P. tectus* showed no marked preference either for foods producing the highest egg output or for those on which development was quickest.

Two inherited variations have been noted—the length of the hind wings, and an emargination of the tip of each elytron. Beetles with long wings were heavier than those with short wings.

The problem of correlating the results of laboratory experiments conducted at constant physical conditions is discussed and a map has been constructed showing the areas where *P. tectus* would be expected to maintain itself. This is compared with the recorded distribution of the species.

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References.

- BARRETT, C. J. (1942). New records of Coleoptera from Offaly (Kings Co.)—Ent. mon. Mag., **78**, pp. 42–43.
- BELTON, C. H. (1951). Note on some insect pests in stored products.—N.Z.J. Sci. Tech., (A) **32** no. 1, p. 44.
- BENTLEY, E. W. (1944). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. V. Humidity reactions.—J. exp. Biol., **20**, pp. 152–158.
- BENTLEY, E. W., GUNN, D. L. & EWER, D. W. (1941). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. I. The daily rhythm of locomotory activity, especially in relation to light and temperature.—J. exp. Biol., **18**, pp. 182–195.
- BIRCH, L. C. (1948). The intrinsic rate of natural increase of an insect population.—J. Anim. Ecol., **17**, pp. 15–26.
- BRAUNE, R. (1943). Vergleichende Untersuchungen an den Diebskäfern *Ptinus tectus* Boield., *Ptinus fur* L., *Ptinus latro* Fabr., *Ptinus seapunctatus* Panz., und *Ptinus brunneus* Duft., zugleich der experimentelle Beweis für die Notwendigkeit des Flüssigkeitsausgleichs im Insektenkörper.—Z. Morph. Ökol. Tiere, **39**, pp. 546–691.
- BRINCK, P. (1945). Coleoptera.—Sci. Res. Norweg. Antarctic Exped., no. 24, 23 pp.
- BROWN, W. J. (1929). Some new species of Coleoptera.—Canad. Ent., **61**, pp. 108–110.
- BROWN, W. J. (1940). A key to the species of Ptinidae occurring in dwellings and warehouses in Canada (Coleoptera).—Canad. Ent., **72**, pp. 115–122.
- CARPENTER, G. H. (1908). Injurious insects and other animals observed in Ireland during the year 1907.—Econ. Proc. R. Dublin Soc., **1**, pp. 559–588.

- CHITTY, A. J. (1904). *Ptinus tectus* and *Lathridius bergrothi* in Holborn.—Ent. mon. Mag., **40**, p. 109.
- CRAWFORD, W. M. (1932). Coleoptera in Northern Ireland.—Ent. mon. Mag., **68**, pp. 277–278.
- DAVID, H. A. (1951). Further applications of range to the analysis of variance.—Biometrika, **38**, pp. 393–407.
- DEAL, J. (1941). The temperature preferendum of certain insects.—J. Anim. Ecol., **10**, pp. 323–356.
- DONISTHORPE, H. (1947). Insects in a pigeon's nest.—Ent. mon. Mag., **83**, p. 294.
- VAN EMDEN, F. (1931). Zur Kenntnis der Morphologie und Oekologie des Brotkäfer-Parasiten *Cephalonomia quadridentata* Duchaussoy.—Z. Morph. Oekol. Tiere, **23**, pp. 425–574.
- EVERTS, E. (1924). Vierde vervolg op het aanhangsel in "Coleoptera Neerlandica" III. (Nieuwe vondsten voor de Nederlandsche Coleopterenfauna XLII.)—Ent. Ber., **6**, pp. 277–285.
- EWER, D. W. & EWER, R. F. (1942). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. III. The effect of temperature and humidity on oviposition, feeding and duration of life cycle.—J. exp. Biol., **18**, pp. 290–305.
- FISHER, R. A. (1946). Statistical methods for research workers.—10th revd. edn. London, Oliver & Boyd.
- FRAENKEL, G. & BLEWETT, M. (1943a). The natural foods and the food requirements of several species of stored products insects.—Trans. R. ent. Soc. Lond., **93**, pp. 457–490.
- FRAENKEL, G. & BLEWETT, M. (1943b). The basic food requirements of several insects.—J. exp. Biol., **20**, pp. 28–34.
- FRAENKEL, G. & BLEWETT, M. (1943c). Vitamins of the B group required by insects.—Nature, **151**, pp. 703–704.
- FRAENKEL, G. & BLEWETT, M. (1943d). The sterol requirements of several insects.—Biochem. J., **37**, pp. 692–695.
- FREEMAN, J. A. (1948). Stored products pests: a survey of the principal entomological problems in the United Kingdom.—Ann. appl. Biol., **35**, pp. 294–301.
- FREEMAN, J. A. (1950). Methods of spread of stored products insects and origin of infestation in stored products.—Proc. 8th int. Congr. Ent., Stockholm 1948, pp. 815–825.
- FRIEDERICH, H. F. (1932). Zur Biologie von *Ptinus tectus* Boield.—Z. angew. Ent., **19**, pp. 301–306.
- GRAY, H. E. (1950). Stored product insect conditions in Canada in 1949.—Canad. Insect Pest Rev., **28**, pp. 96–97.
- GUNN, D. L. & HOPF, H. S. (1942). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. II. The amount of locomotory activity in relation to experimental and to previous temperatures.—J. exp. Biol., **18**, pp. 278–289.
- GUNN, D. L. & KNIGHT, R. H. (1945). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. VI. Culture conditions.—J. exp. Biol., **21**, pp. 132–143.
- GUNN, D. L. & WALSHE, B. M. (1942). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. IV. Temperature preference.—J. exp. Biol., **19**, pp. 133–140.
- HANSEN, V. (1923). Tillaeg og Rettelser til vore Billerfortegnelser.—Ent. Medd., **14**, pp. 240–242.

- HATCH, M. H. (1933). *Ptinus tectus* Boieldieu in America.—Bull. Brooklyn ent. Soc., **28**, pp. 200–202.
- HATCH, M. H. (1943). *Ptinus tectus* damaging furs in Alaska.—J. econ. Ent., **36**, p. 353.
- HAVELKA, J. (1945). Příspěvek k poznání výskytu *Ptinus* (*Pseudobruchus*) *tectus* Boield. v naší fauně.—Věda Přír., **23**, pp. 181–182.
- HAYHURST, H. (1940). Insect pests in stored products.—83 pp. London, Chapman & Hall.
- HELLÉN, W. (1925). Uebersicht der Ptiniden (Col.) Finlands.—Not. Ent., Helsingfors, **5**, pp. 118–123.
- HENDERSON, J. L. (1945). The beetles of a suburban London garden in Surrey.—Ent. mon. Mag., **81**, pp. 63–66.
- HICKIN, N. E. (1941). Notes on mites found associated with cultures of Ptinid beetles.—Ent. mon. Mag., **77**, p. 197.
- HICKIN, N. E. (1942a). The food and water requirements of *Ptinus tectus* Boieldieu (Coleopt., Ptinidae).—Proc. R. ent. Soc. Lond., (A) **17**, pp. 99–108.
- HICKIN, N. E. (1942b). Infestation of foodstuffs by *Ptinus tectus* Boield. (Col., Ptinidae).—Ent. mon. Mag., **78**, p. 14.
- HINTON, H. E. (1941). The Ptinidae of economic importance.—Bull. ent. Res., **31**, pp. 331–381.
- HOWE, R. W. (1949a). Studies on beetles of the family Ptinidae. 1.—Notes on the biology of species in Britain.—Ent. mon. Mag., **85**, pp. 137–139.
- HOWE, R. W. (1949b). Studies on beetles of the family Ptinidae. 2.—Fineness of foodstuffs as a factor influencing the rate of development of *Ptinus tectus* Boield.—Ent. mon. Mag., **85**, pp. 189–190.
- HOWE, R. W. (1950a). Studies on beetles of the family Ptinidae. III.—A two-year study of the distribution and abundance of *Ptinus tectus* Boield. in a warehouse.—Bull. ent. Res., **41**, pp. 371–394.
- HOWE, R. W. (1950b). Studies on beetles of the family Ptinidae. 4.—A note on an anomalous effect of parental age on the speed of development.—Ent. mon. Mag., **86**, pp. 325–326.
- HOWE, R. W. (1952). Entomological problems of food storage in northern Nigeria.—Bull. ent. Res., **43**, pp. 111–144.
- HOWE, R. W. (1953). Studies on beetles of the family Ptinidae. VIII. The intrinsic rate of increase of some Ptinid beetles.—Ann. appl. Biol., **40**, pp. 121–133.
- HOWE, R. W. & BURGESS, H. D. (1951). Studies on beetles of the family Ptinidae. VI.—The biology of *Ptinus fur* (L.) and *P. sexpunctatus* Panzer.—Bull. ent. Res., **42**, pp. 449–511.
- HOWE, R. W. & BURGESS, H. D. (1952). Studies on beetles of the family Ptinidae. VII.—The biology of five Ptinid species found in stored products.—Bull. ent. Res., **43**, pp. 153–186.
- KEMPER, H. (1938). Hausschädlinge als Bewohner von Vogelnestern.—Z. hyg. Zool., **30**, pp. 227–236, 269–274, 291–297.
- KÖNIG, W. (1936). Biologische Studien über *Ptinus tectus* Boield.—Z. wiss. Zool., **148**, pp. 556–599.
- VON LENDERKEN, H. (1929). Zur Lebensweise und zur Frage der Schadwirkung von *Ptinus tectus* Boield.—Mitt. Ges. Vorratsschutz, **5**, no. 2, pp. 21–26.
- LEPIGRE, A. L. (1951). Insectes du logis et du magasin.—339 pp. Algiers, Insectarium, Jardin d'Essai.
- LINSLEY, E. G. & MICHELbacher, A. E. (1943). A report on insect infestation of stored grain in California.—J. econ. Ent., **36**, pp. 829–831.

- MANSBRIDGE, G. H. (1936). A note on the resistance to prolonged cold of some insect pests of stored products.—Proc. R. ent. Soc. Lond., (A) **11**, pp. 83–86.
- MANTON, S. M. (1945). The larvae of the Ptinidae associated with stored products.—Bull. ent. Res., **35**, pp. 341–365.
- MATHER, K. (1949). Statistical analysis in biology.—3rd edn. London, Methuen.
- MAYNÉ, R. (1948). Report on insects, mites and other pests harmful to stored grains and flours in Belgium.—In Easter, S.S. *Ed.* Preservation of Grains in Storage.—FAO agric. Stud., no. 2, pp. 72–78.
- MUSGRAVE, A. J. (1946). Proceedings of the general meeting held on the 4th December, 1946.—Proc. R. ent. Soc. Lond., (C) **11**, p. 45.
- RICHARDS, O. W. & HERFORD, G. V. B. (1930). Insects found associated with cacao, spices and dried fruits in London warehouses.—Ann. appl. Biol., **17**, pp. 367–395.
- SCHOLTZ, M. F. R. (1920). Die Aufzucht von *Ptinus tectus* Boield.—Ent. Bl., **16**, pp. 23–24.
- SHAPIRO, L. (1941). [The Australian spider beetle] (*Ptinus tectus* Boield.).—Sprav. Vop. Karant. Rast., **3**, pp. 1–4. [In Russian.]
- SOLOMON, M. E. (1951). Control of humidity with potassium hydroxide, sulphuric acid, or other solutions.—Bull. ent. Res., **42**, pp. 543–554.
- SPOON, W. & LOOSJES, F. E. (1947). Waarnemingen over de bescherming die de meerwandige kraftpapierenzak biedt tegen voorraad-insecten.—Papierwereld, **1**, p. 296.
- WADDINGTON, C. H. (1942). The development of rudimentary wings in *Ptinus tectus* Boield. (Coleoptera: family Ptinidae).—Proc. zool. Soc. Lond., (A) **112–113**, pp. 13–20.
- WILSON, H. F. (1915). Insect pests of stored products.—Bienn. Rep. Ore. agric. Exp. Sta., 1913–14, pp. 127–130.
- WOODROFFE, G. E. & SOUTHGATE, B. J. (1951). Birds' nests as a source of domestic pests.—Proc. zool. Soc. Lond., **121**, pp. 55–62.
- ZACHER, F. (1927). Die Vorrats-, Speicher- und Materialschädlinge und ihre Bekämpfung.—366 pp. Berlin, Parey.
- ZACHER, F. (1933). Haltung und Züchtung von Vorratsschädlingen. In Abderhalden. Handb. biol. Arb. Meth., Abt. IX. Teil 7, (Lief. 416), pp. 389–592.
- ZACHER, F. (1939a). Schädlingsverbreitung und Nahrungsmittelhandel.—Dtsch. Lebensmitt.-Rdsch., **1939**, no. 9, repr. 4 pp.
- ZACHER, F. (1939b). Verschleppung und Einbürgerung von Vorratsschädlingen.—Verh. 7. int. Kongr. Ent., Berlin, **4**, pp. 2919–2926.
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FIG. 1.



FIG. 2.

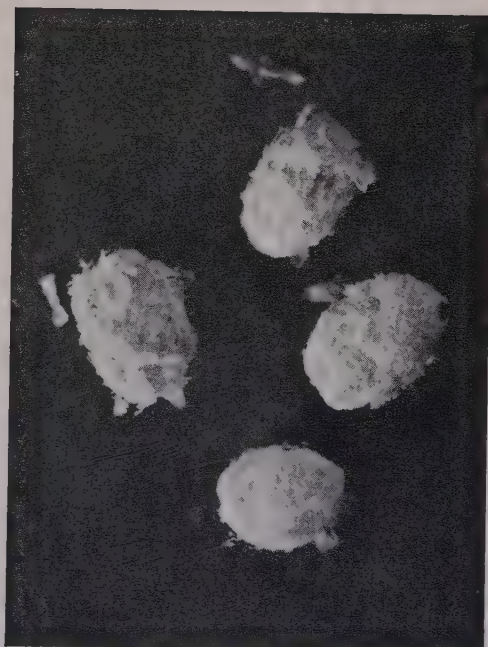


FIG. 3.



FIG. 4.

The immature stages of *P. tectus*. FIG. 1. Eggs (X 30); FIG. 2. Larvae (X 8); FIG. 3. Cocoons (X 5); FIG. 4. Pupae (X 7).

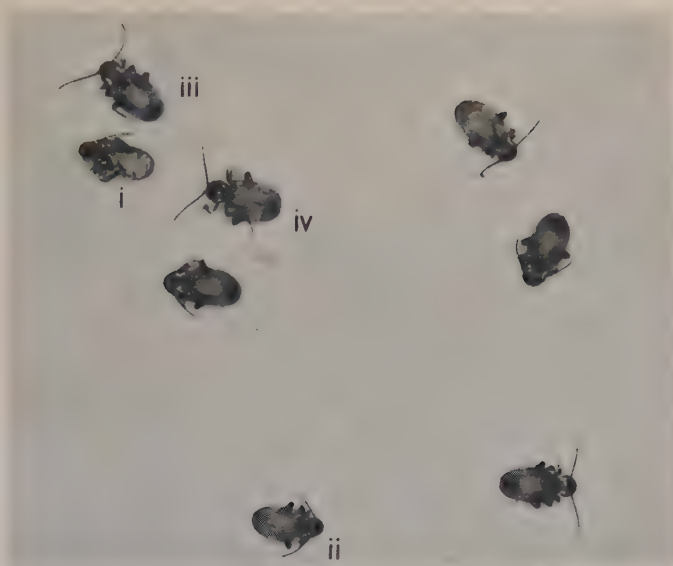


FIG. 1. Beetles photographed immediately after being disturbed, showing typical positions of the death-feigning attitude and one beetle (iv) which has not reacted and is still moving.

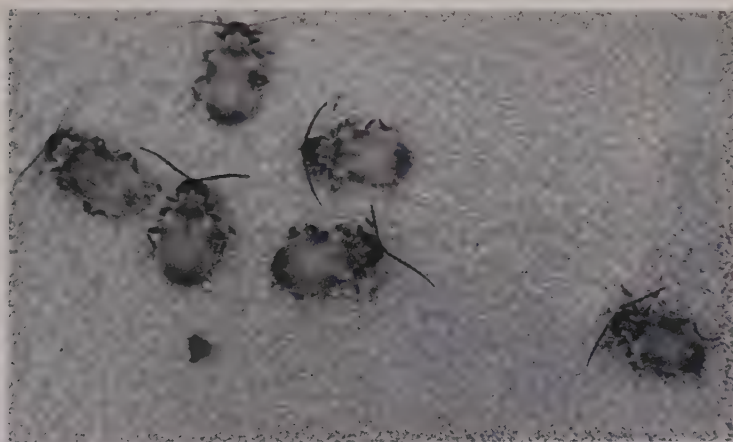


FIG. 2. Thirsty beetles drinking on *wet* blotting paper on which they have just been placed.



FIG. 3. Similar beetles running over *dry* blotting paper.

SOME NOTES ON THE RECORDED DISTRIBUTION OF OLD WORLD SPECIES OF *ORNITHODOROS* (ACARINA).

By H. S. LEESON.

London School of Hygiene and Tropical Medicine.

Leeson

These notes are the result of an examination of the literature and are concerned with geographical distribution. The *Review of Applied Entomology*, Series B, and the *Tropical Diseases Bulletin* were consulted and many papers were seen in the original; references to a selection of the more important of these is appended herewith. Of the ticks of the genus *Ornithodoros* occurring in the Old World about 40 names appear in published papers; of these about 20 seem to be valid species and five are well known and readily recognised: *erraticus*, *lahorensis*, *moubata*, *savignyi* and *tholozani*. Most of the Old World

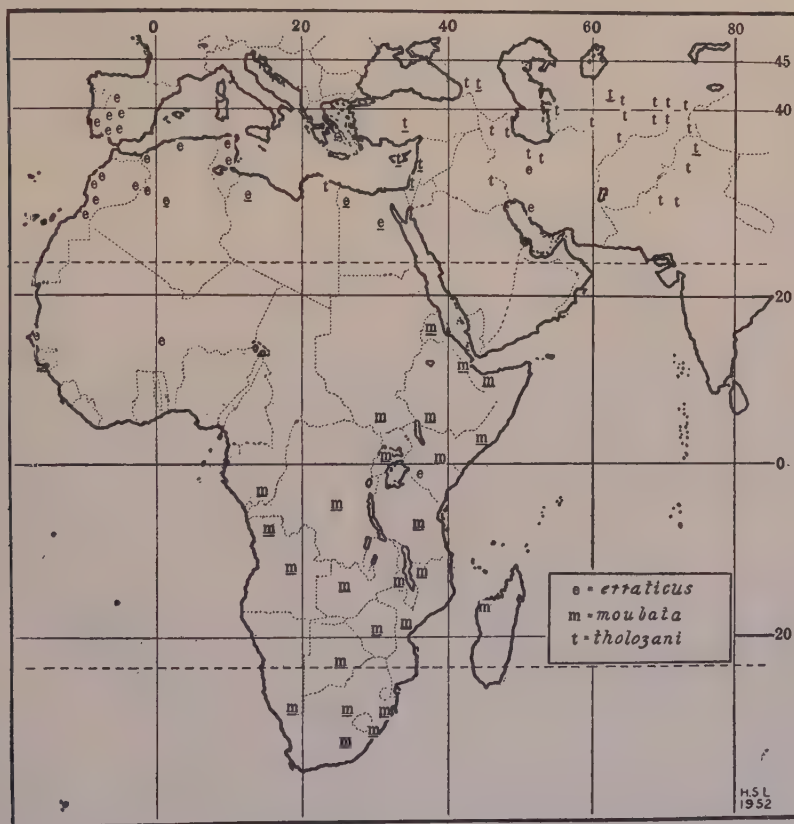


Fig. 1.—Distribution of *Ornithodoros erraticus*, *O. moubata* and *O. tholozani*. In French Sudan the form of *O. erraticus* is the variety *sonrai*.

species have been reported from countries lying between latitudes 45°N. and 35°S. and longitudes 20°W. and 90°E. Outside these limits the only species recorded are *capensis* in Tristan da Cunha, *batuensis* in Malaya and *gurneyi* in Australia; in addition, it is reported that another as yet undescribed species near *batuensis* has been discovered in the Philippines (Hoogstraal, personal communication, 1952).

In the following list of species, the synonyms quoted are those generally accepted by modern workers, and the authority for the synonymy is given in brackets. The name of the country in which the species was originally found is printed first in capitals; the names of other countries where the species has since been recorded follow in alphabetical order.

On the accompanying maps of distribution (figs. 1-4), underlined letters refer to records for a country or similar large area. Other letters are placed approximately over the locality recorded. In building up the maps it was remembered that "a report of a species in an area remote from its known range of distribution is open to question" (Cooley, 1942).



Fig. 2.—Distribution of *Ornithodoros capensis*, *O. lahorensis* and *O. savignyi*. The letter "S" in South West Africa may also be taken to indicate the presence of *O. pavementosus* which some authors regard as a form of *O. savignyi*.

THE SPECIES OF *ORNITHODOROS* RECORDED IN THE LITERATURE AS
OCCURRING IN COUNTRIES OF THE OLD WORLD.

asperus (see *tholozani*).

batuensis Hirst, 1929.

MALAYA.

Hoogstraal (personal communication, 1952) informs me that a new species near *batuensis* has been observed in the Philippines.

canestrinii (Birula) 1895.

PERSIA; Azerbaijan; Daghestan.

Brumpt (1935) recorded the first find of *canestrinii* in Persia since the tick was described.

capensis Neumann, 1901.

CAPE PROVINCE, South Africa; Tristan da Cunha.

cholodkovskyi Pavlovsky, 1930.

TURKMENISTAN.

coniceps Canestrini, 1890.

ITALY; France; ? Morocco; Palestine; Tunisia.

Brumpt (1936b) regards *coniceps* Canestrini as a distinct species and not as a variety of *talaje*; the morphology and geographical distribution of the two species are different. It is also probable that the record of Martial and Senevet (1921), of *talaje* in Morocco, refers to *coniceps* Canestrini.

coniceps Berlese, 1890 (see *savignyi*).

crossi (see *tholozani* var. *crossi*).

delanoei Roubaud & Colas-Belcour, 1931.

MOROCCO.

delanoei subsp. *acinus* Whittick, 1938.

BRITISH SOMALILAND.

erraticus Lucas, 1846.

Synonyms: *miliaris* Karsch, 1880 (Neumann, 1901); *maroccanus* Velu, 1919 (Nicolle, Anderson and Colas-Belcour, 1929).

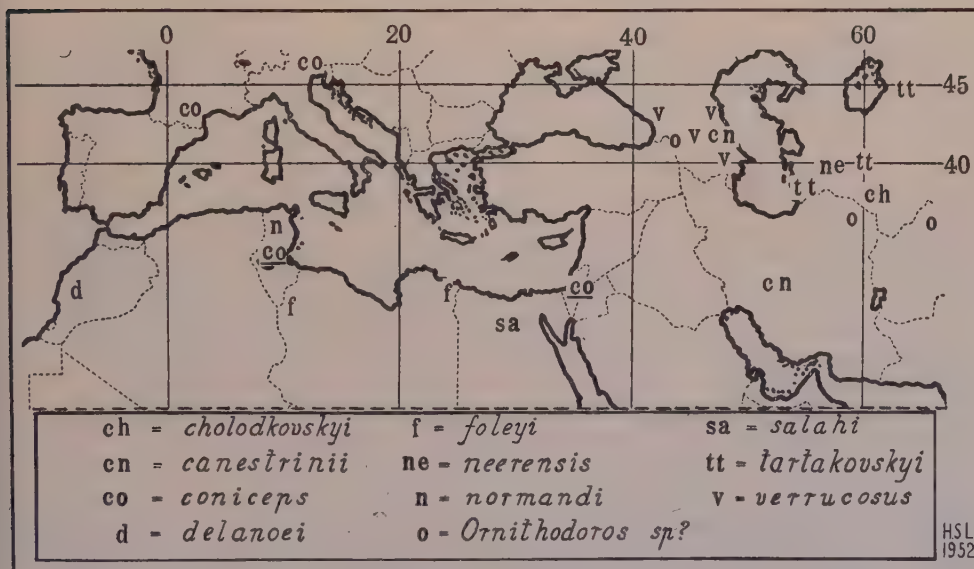


Fig. 3.—Distribution of *Ornithodoros cholodkovskyi*, *O. canestrinii*, *O. coniceps*, *O. delanoei*, *O. foleyi*, *O. neerensis*, *O. normandi*, *O. salahi*, *O. tartakovskiy* and *O. verrucosus*.

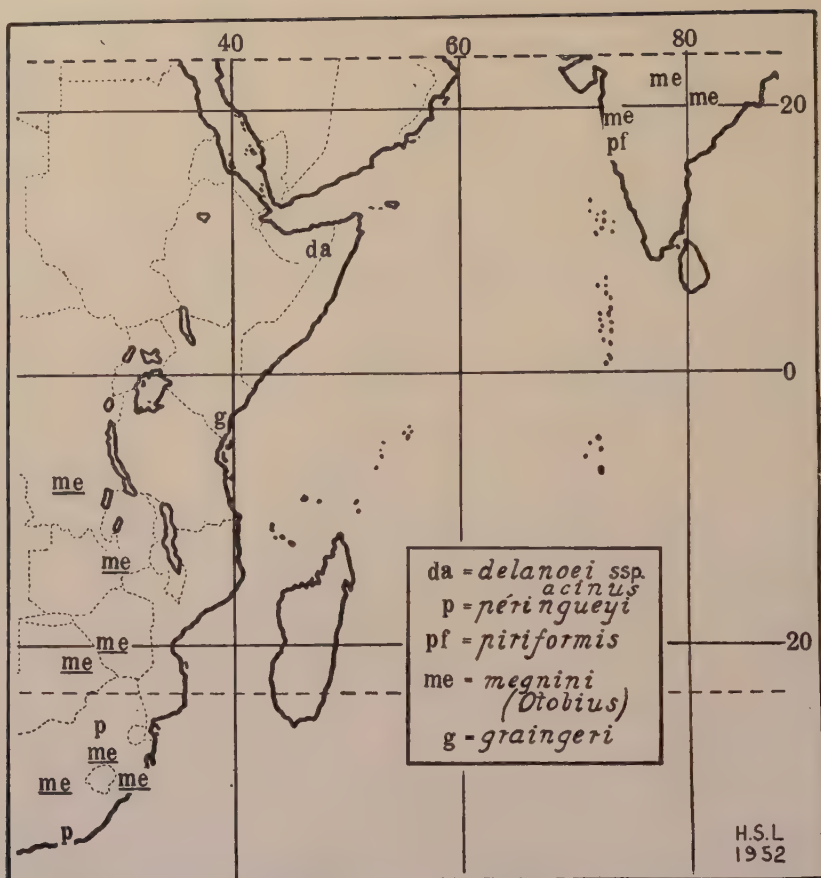


Fig. 4.—Distribution of *Ornithodoros delanoei* subsp. *acinus*, *O. graingeri*, *O. pèringueyi*, *O. piriformis* and *Otobius megnini*. The subspecies *acinus* has been included because it occurs (in Somaliland) so far from the type locality (Morocco), and *Otobius megnini* because of its veterinary interest.

ALGERIA; Egypt; French Sudan; Kenya; Morocco; Persia; Portugal; Senegal; Spain; Tunisia.

De Carvalho Dias (1937) gives the distribution of *erraticus* in Portugal. Gil Collado (1938) gives the distribution of *erraticus* in Spain. Heisch and Grainger (1950) record *erraticus* in Kenya. Blanc, Chabaud and Bruneau (1951) record different sizes of *erraticus* in different areas in Morocco. Hoogstraal (personal communication, 1952) reports *erraticus* as being very common in Egypt, even in Cairo.

erraticus var. *sonrai* Sautet & Witkowski, 1944.

FRENCH SUDAN.

foleyi Parrot, 1928.

Synonym: *franchinii* Tonelli-Rondelli, 1930 (Brumpt, 1936b).

ALGERIA: SAHARA; Libya; Cyrenaica; Tripolitania.

franchinii (see *foleyi*).

graingeri Heisch & Guggisberg, 1953.

KENYA (Diani).

gurneyi Warburton, 1926.

AUSTRALIA: New South Wales, Queensland.
lahorensis Neumann, 1908.

INDIAN SUBCONTINENT: LAHORE; Armenia; Azerbaijan; Georgia; Jugoslavia; Kashmir; Kazakstan; Palestine; Persia; Tibet; Turkey (Asia Minor); Turkmenistan; Uzbekistan.

Olenov (1935) records the most north-easterly occurrence of a species of *Ornithodoros*, that of *lahorensis* at Katu-tau mountains, Kazakstan (between 44° and 45°N. and 78° and 80°E.). According to Pavlov (1944) *lahorensis* is the only *Ornithodoros* species in the Balkans.

maroccanus (see *erraticus*).

megnini (Dugès) 1883.

This species was removed from *Ornithodoros* to the genus *Otobius* by Banks (1912).

An American species which now appears to be established in Belgian Congo; Indian subcontinent: Bombay, Central; Rhodesia: Northern and Southern; Union of South Africa: Cape Province, Natal, Orange Free State, Transvaal.

miana (see *tholozani*).

miliaris (see *erraticus*).

morbillosus (see *savignyi*).

moubata (Murray) 1877.

Synonym: *savignyi* var. *caeca* Neumann 1901 (Nuttall & others, 1908-15).

ANGOLA; Abyssinia; Bechuanaland; Congo: Belgian, Middle (south); Eritrea; Kenya; Madagascar; Mozambique; Nyasaland; Rhodesia: Northern and Southern; Somaliland: British, French, Italian; South-West Africa; Sudan, Anglo-Egyptian (South); Tanganyika; Uganda; Union of South Africa: Cape Province, Natal, Transvaal, Zululand.

Distribution summarised by Leeson (1952). Vanderplank (personal communication, 1952) informs me that *moubata* is "extremely rare (in Zanzibar and Pemba) if present at all, but odd individuals are imported ex dhows from Tanganyika."

neerensis Pavlovsky, 1941.

TURKMENISTAN.

normandi Larrousse, 1923.

TUNISIA.

Often accompanied by *erraticus*.

papillipes (see *tholozani*).

pavimentosus Neumann, 1901.

SOUTH-WEST AFRICA.

péringueyi Bedford & Hewitt, 1925.

CAPE PROVINCE (South Africa); Transvaal.

piriformis Warburton, 1918.

INDIAN SUBCONTINENT: Bombay.

salahi Hoogstraal, 1953.

EGYPT.

savignyi (Audouin) 1826.

Synonyms: *morbillosus* Gerstaecker, 1873 (Nuttall & others, 1908-15); *schinzii* (Berlese) 1889 (Nuttall & others, 1908-15).

EGYPT; Abyssinia; Algeria; Arabia: Aden, Yemen; Bechuanaland; Ceylon; Congo: Belgian; Eritrea; Indian subcontinent: Punjab, south-east; Iraq; Israel; Kenya; Libya: Cyrenaica, Tripolitania; Mozambique; Northern Nigeria; Somaliland: British, French, Italian; South-West Africa; Sudan: Anglo-Egyptian (north), French; Tanganyika; Tunisia; Union of South Africa: Cape Province, Transvaal; Uganda.

Lounsbury (1899-1903) writing of ticks in South Africa referred to the "tampan", "*Ornithodoros*", and to "*Ornithodoros savignyi*" but never to *O. moubata*. He made no reference to the presence of eyes or to their absence. Corson and Ingram (1923) mention that *O. savignyi* is absent from the Gold Coast. Brumpt (1936a) records the most westerly locality for *savignyi* as Azaouad, Timbuctoo. The same author recorded *savignyi* from Ceylon for the first time. Kirk (1939) says the Argasid in the northern part of the Anglo-Egyptian Sudan is *O. savignyi*, that in the south is *O. moubata*. Anderson (1947) states that *savignyi* is not found in houses and coffee shops in British Somaliland but in the open. Heisch (1950) has never found *savignyi* in native huts. Browning (personal communication, 1951) states that Nuttall's collection, now in the British Museum, includes a single female labelled *O. savignyi* from Lounsbury, South Africa and dated v. 1906 and that it is in fact *savignyi* and not *moubata*. Also in this collection is another female from Transvaal, labelled C. W. Howard, 4. vi. 1909 and this is also correctly named *savignyi*. Gambles (personal communication, 1951) records the presence of *savignyi* in cattle markets in northern Nigeria and the absence of *moubata*. Walton (1951) reported the occurrence of *savignyi* for the first time south of Buna and Dela in the Northern Province of Kenya. Zumpt (personal communication, 1951) says he has seen *savignyi* "from several localities in South-West Africa including Karasberg, Epukiro, Aminuis, Ondanqua, Ovitoto and Gobabis."

Considerable research has failed to discover the species that is referred to by Brumpt in his "Précis de Parasitologie" 1949, 6th edition, Vol. 2, page 1126, as *Argas coniceps* Berlese 1890, therefore it has been omitted from the synonyms of *Ornithodoros savignyi* Audouin 1826 rather than perpetuate a possible error.

savignyi var. *caeca* (see *moubata*).

schinzii (see *savignyi*).

talaje (Guérin-Ménéville) 1849.

An American tick. Martial and Senevet (1921) record *talaje* at Fez, Morocco, but seem uncertain of their identification. Brumpt (1936b) says Martial and Senevet's tick is probably *coniceps* Canestrini. Corson and Ingram (1923) remark that *talaje* is very rare in the Gold Coast.

talaje var. *capensis* (see *capensis*).

talaje var. *coniceps* (see *coniceps* Canestrini).

tartakovskyi Olenov, 1931.

UZBEKISTAN; Kazakstan; Turkmenistan.

tholozani (Laboulbène & Mégnin) 1882.

Synonyms: *asperus* Warburton, 1918 (Desportes & Campana, 1946); *papillipes* Birula, 1895 (Neumann, 1901); *miana* Starobynsky, 1922 (nom. nud.).

PERSIA; Azerbaijan; Cyprus; Daghestan; Georgia; Indian subcontinent; Lahore; Iraq; Israel; Kashmir; Kazakstan; Kirghizia; Lebanon; Libya; Cyrenaica; Syria; Tadzikistan; Turkey; Turkmenistan; Uzbekistan.

Desportes and Campana (1946) regard the specimens collected by Brumpt (1939) in Babylon and considered by him to be *asperus* Warburton as identical with the typical *tholozani* of Persia and Asia Minor; the name *asperus* they consider to be of doubtful validity. They describe the form found in Central Asia as a new variety of *tholozani* and name it *pavlovskyi*. Starobynsky (1922) refers to a tick "*O. miana*" being responsible for the transmission of Persian Relapsing Fever. This tick seems never to have been described and is probably *tholozani*. Coghill, Lawrence and Ballantine (1947) had certain ticks from Tobruk identified as *tholozani*; this locality is the most westerly point of its distribution.

tholozani var. *crossi* Brumpt, 1921.

INDIAN SUBCONTINENT: Punjab.

There is some confusion about the status of the name "*crossi*." Up to now (November, 1952) I have been unable to find the original description of *Ornithodoros crossi*, if, indeed, one were ever published. Chabaud (personal communication, 1952) informs me that he knows of none. The facts are these: Cross and Patel (1921) stated that the name *crossi* was proposed by Brumpt for a new tick they had found in the Punjab. In 1922 Cross and Patel repeated this statement. Brumpt (1922) gave the name *Ornithodoros Grossi* Brumpt 1921 (spelt with a capital "G" not "c") on page 775 of his *Précis de Parasitologie*, 3rd edition, but no description. He mentioned that it was discovered in India by H. E. Cross. Specimens identified by Pavlovsky (1930) as *papillipes* (after comparison with Birula's type-material), were sent by him to Nuttall and Warburton who found them to be *crossi*. The ticks were then sent by Nuttall and Warburton to Brumpt who agreed that they and *crossi* were one and the same species. Pavlovsky (1930) therefore made *crossi* a synonym of *papillipes*. Then Brumpt (1934) regarded *crossi* as a synonym of *papillipes* and later Brumpt (1936b) gave *papillipes* and *crossi* as synonyms of *tholozani*. But Neumann (1901) had already made *papillipes* a synonym of *tholozani*. In 1944 Sapre published a full description of a tick which he regarded as *crossi* because, as he says, Brumpt's (1936b) description was inadequate (I have not been able to trace this 1936 description). Sapre in the same paper gives a table of characters distinguishing *tholozani*, *cholodkovskyi* and the Russian and Indian forms of *papillipes* and states that the Indian form of *papillipes* is a distinct species identical with *crossi* Brumpt 1921. Desportes and Campana (1946) called the Indian tick *Ornithodoros tholozani* var. *crossi* and Rao and Kalra (1949) sent specimens of it to Kohls who also thinks this form should be called *Ornithodoros tholozani* var. *crossi*.

tholozani var. *pavlovskyi* Desportes & Campana, 1946.

CENTRAL ASIA (see *tholozani*).

verrucosus Olenov, Zasukhin & Fenyuk, 1934.

GEORGIA; Azerbaijan; Daghestan.

SUMMARY OF THE DISTRIBUTION OF OLD WORLD SPECIES OF ORNITHODOROS.

Names enclosed in brackets, e.g. (*megnini*) are species of New World origin.

ABYSSINIA: *moubata*, *savignyi*.

ADEN: see ARABIA.

AFRICA: South-west, *moubata*, *pavimentosus*, *savignyi*.

ALGERIA: *erraticus*, *savignyi*; Sahara, *foleyi*, *savignyi*.

ANGOLA: *moubata*.

ARABIA: Aden, Yemen, *savignyi*.

ARMENIA: *lahorensis*.

ASIA: Central, *tholozani* var. *pavlovskyi*.

AUSTRALIA: New South Wales, Queensland, *gurneyi*.

AZERBAIJAN: *canestrinii*, *lahorensis*, *tholozani*, *verrucosus*.

BECHUANALAND: *moubata*, *savignyi*.

CAUCASUS: *canestrinii*, *verrucosus*.

CEYLON: *savignyi*.

CONGO: Belgian, (*megnini*), *moubata*, *savignyi*; Middle, *moubata*; Portuguese, *moubata*.

CYPRUS: *tholozani*.

CYRENAICA: see LIBYA.

DAGHESTAN: *canestrinii*, *tholozani*, *verrucosus*.

EGYPT: *erraticus*, *savignyi*, *salahi*.

ERITREA: *moubata*, *savignyi*.

- FRANCE: *coniceps*.
 GEORGIA: *lahorensis*, *tholozani*, *verrucosus*.
 GOLD COAST: (?*talaje*).
 GREAT NAMAQUALAND: see AFRICA, South-west.
 INDIAN SUBCONTINENT: Bombay, (*megnini*), *piriformis*; Central, (*megnini*); Lahore, *lahorensis*, *tholozani*; Punjab, *lahorensis*, *savignyi*, *tholozani*, *tholozani* var. *crossi*; south, *savignyi*; south-east, *savignyi*.
 IRAK: *tholozani*, *savignyi*.
 ISRAEL: *coniceps*, *lahorensis*, *savignyi*, *tholozani*.
 ITALY: *coniceps*.
 JUGOSLAVIA: *lahorensis*.
 KASHMIR: *lahorensis*, *tholozani*.
 KAZAKSTAN: north-east, *lahorensis*; south, *tartakovskiy*, *tholozani*.
 KENYA: *erraticus*, *graingeri*, *moubata*, *savignyi*.
 KIRGHIZIA: *tholozani*.
 LEBANON: *tholozani*.
 LIBYA: south, *savignyi*; Cyrenaica, *foleyi*, *savignyi*, *tholozani*; Tripolitania, *foleyi*, *savignyi*.
 MADAGASCAR: *moubata*.
 MALAYA: *batuensis*.
 MOROCCO: *delanoei*, *erraticus*, ?*coniceps*.
 MOZAMBIQUE: *moubata*, *savignyi*.
 NAMAQUALAND: see AFRICA, South-west.
 NIGERIA: Northern Province, *savignyi*.
 NYASALAND: *moubata*.
 PALESTINE: see ISRAEL.
 PERSIA: *canestrinii*, *erraticus*, *lahorensis*, *tholozani*.
 PHILIPPINES: n.sp. near *batuensis*.
 PORTUGAL: *erraticus*.
 RHODESIA: Northern, (*megnini*), *moubata*, *savignyi*; Southern, (*megnini*), *moubata*.
 SENEGAL: *erraticus*.
 SOMALILAND: British, *delanoei* var. *acinus*, *moubata*, *savignyi*; French, *savignyi*; ITALIAN, *moubata*, *savignyi*.
 SPAIN: *erraticus*.
 SUDAN: Anglo-Egyptian, north, *savignyi*; south, *moubata*; French, *erraticus*, *erraticus* var. *sonrai*, *savignyi*.
 SYRIA: *tholozani*.
 TADZIKISTAN: *tholozani*.
 TANGANYIKA: *moubata*, *savignyi*.
 TIBET: *lahorensis*.
 TRIPOLITANIA: see LIBYA.
 TRISTAN DA CUNHA: *capensis*.
 TUNISIA: *erraticus*, *normandi*, *savignyi*; Jerba, *savignyi*.
 TURKESTAN: *cholodkovskiy*, *lahorensis*, *tholozani*.
 TURKEY: Asia Minor, *lahorensis*, *tholozani*.
 TURKMENISTAN: south and south-west, *cholodkovskiy*, *lahorensis*, *neerensis*, *tartakovskiy*, *tholozani*.
 UGANDA: *moubata*, *savignyi*.
 UNION OF SOUTH AFRICA: Cape Province, *capensis*, (*megnini*), *moubata*, *péringueyi*, *savignyi*; Natal, (*megnini*), *moubata*; Orange Free State, (*megnini*); Transvaal, (*megnini*), *moubata*, *péringueyi*, *savignyi*; Zululand, *moubata*.
 UZBEKISTAN: *lahorensis*, *tholozani*, *tartakovskiy*.
 YEMEN: see ARABIA.

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References.

- ANDERSON, T. F. (1947). *E. Afr. med. J.*, **24**, pp. 259-261.
- AUDOUIN, V. (1826). *Egypte*, 2nd edn., **22**, p. 182.
- BANKS, N. (1912). *Proc. ent. Soc. Wash.*, **14**, pp. 96-99.
- BEDFORD, G. A. H. (1934). *Onderstepoort J. vet. Sci.*, **2**, pp. 49-99.
- BEDFORD, G. A. H. & HEWITT, J. (1925). *S. Afr. J. nat. Hist.*, **5**, pp. 259-266.
- BERLESE, A. (1889). *Atti Soc. Veneto-Trent.*, **10**, p. 289.
- BIRULA, A. (1895). *Bull. Acad. Sci. St. Petersburg*, **5**, p. 353.
- BLANC, G., CHABAUD, A. G. & BRUNEAU, J. (1951). *Arch. Inst. Pasteur Maroc*, **4**, pp. 354-359.
- BRUMPT, E. (1922). *Précis de Parasitologie*, 3rd edn. Paris, Masson.
- BRUMPT, E. (1934). *Bull. Soc. Path. exot.*, **27**, pp. 510-512.
- BRUMPT, E. (1935). *Bull. Soc. Path. exot.*, **28**, pp. 51-53.
- BRUMPT, E. (1936a). *Ann. Parasit. hum. comp.*, **14**, pp. 632-639, 640-646.
- BRUMPT, E. (1936b). *Précis de Parasitologie*, **2**, 5th edn. Paris, Masson.
- BRUMPT, E. (1939). *C. R. Acad. Sci., Paris*, **208**, pp. 2029-2031.
- BRUMPT, E. (1949). *Précis de Parasitologie*, **2**, 6th edn. Paris, Masson.
- CANESTRINI, G. (1890). *Prospetto dell'Acarofauna italiana*, **4**, p. 535.
- COGHILL, N. F., LAWRENCE, J. & BALLANTINE, I. D. (1947). *Brit. med. J.*, 10th May, 1947, pp. 637-640.
- COOLEY, R. A. (1942). *Publ. Amer. Ass. Advanc. Sci.*, **18**, pp. 77-84.
- CORSON, J. F. & INGRAM, A. (1923). *Rep. med. Dep. Gold Coast, 1922-23*, pp. 28-29, 65-77.
- CROSS, H. E. & PATEL, P. G. (1921). *Vet. Bull. Punjab Dep. Agric.*, no. 6.
- CROSS, H. E. & PATEL, P. G. (1922). *Vet. Bull. Punjab Dep. Agric.*, no. 9.
- DE CARVALHO DIAS, A. (1937). *XII Int. Congr. Zool. Lisbonne 1935*, **3**, pp. 2079-2083.
- DESPORTES, C. & CAMPANA, Y. (1946). *Ann. Parasit. hum. comp.*, **21**, pp. 74-88.
- DUGÈS, A. (1883). *Naturelleza*, **5**, p. 195.
- GERSTAECKER, C. E. A. (1873). *Decken's Reisen in Ostafrika*, **3** (2), p. 464.
- GIL COLLADO, J. (1938). *Broteria, Cienc. nat. (N. S.)*, **7**, pp. 99-109.
- GUÉRIN-MÉNÉVILLE, F. E. (1849). *Mag. Zool.*, (2) **7**, p. 342, pl. 6.
- HEISCH, R. B. (1950). *Ann. trop. Med. Parasit.*, **44**, pp. 260-272.
- HEISCH, R. B. & GRAINGER, E. E. (1950). *Ann. trop. Med. Parasit.*, **44**, pp. 153-155.
- HEISCH, R. B. & GUGGISBERG, C. A. W. (1953). *Parasitology*, **42**, pp. 192-198.
- HIRST, A. S. (1929). *J. F. M. S. Mus.*, **14**, p. 365.
- HOOGSTRAAL, H. (1953). *J. Parasit.*, **39**, pp. 256-263.
- HOWARD, C. W. (1908). *Ann. Transvaal Mus.*, **1**, pp. 73-172.
- KARSCH, F. (1880). *Mitt. münchn. Ent. Ver.*, **4**, p. 141.
- KIRK, R. (1939). *Ann. trop. Med. Parasit.*, **33**, pp. 125-140.
- LABOULBÈNE, J. & MÉGNIN, J. (1882). *J. Anat. Physiol.*, **28**, p. 317.
- LARROUSSE, F. (1923). *Ann. Parasit. hum. comp.*, **1**, pp. 170-177.
- LEESON, H. S. (1952). *Bull. ent. Res.*, **43**, pp. 407-411.
- LOUNSBURY, C. P. (1899a). *Agric. J. C. G. H.*, **15**, pp. 421-422.

- LOUNSBURY, C. P. (1899b). *Agric. J. C. G. H.*, **15**, p. 240.
- LOUNSBURY, C. P. (1900a). *Canad. Ent.*, **32**, pp. 17-24.
- LOUNSBURY, C. P. (1900b). *Rep. Ent. C. G. H.*, 1899, pp. 19-34.
- LOUNSBURY, C. P. (1900c). *Bull. U. S. Bur. Ent.*, no. 26, pp. 41-48.
- LOUNSBURY, C. P. (1902). *Rep. Ent. C. G. H.*, 1901.
- LOUNSBURY, C. P. (1903). *Rep. Ent. C. G. H.* 1902, p. 42.
- LUCAS, H. (1846). *Explor. Algérie*, **1** (1), p. 316.
- MARTIAL, R. & SENEVET, G. (1921). *Bull. Soc. Path. exot.*, **14**, pp. 24-26.
- MURRAY, A. (1877). *Economic Entomology; Aptera, Ixodoidea*, p. 180.
- NEUMANN, L. G. (1901). *Mém. Soc. zool. Fr.*, **14**, pp. 256, 257, 258.
- NEUMANN, L. G. (1908). *J. trop. vet. Sci., Calcutta*, **3**, p. 462.
- NICOLLE, C., ANDERSON, C. & COLAS-BELCOUR, J. (1929). *C. R. Acad. Sci., Paris*, **189**, pp. 1220-1221.
- NUTTALL, G. H. F., WARBURTON, C., COOPER, W. F. & ROBINSON, L. E. (1908/15). *Ticks, a monograph of the Ixodoidea*. Cambridge Univ. Pr.
- OLENEV, N. O. (1931). *Z. Parasitenk.*, **4**, pp. 126-139.
- OLENEV, N. O. (1935). *Trav. Inst. Méd. vét. exp. U. R. S. S.*, **11**, pp. 133-135.
- OLENEV, N. O., ZASUKHIN, D. N. & FENYUK, B. K. (1934). *Rev. Microbiol., Saratov*, **13**, pp. 327-330.
- PARROT, L. (1928). *Bull. Soc. Path. exot.*, **21**, pp. 520-524.
- PAVLOV, P. (1944). *Z. Parasitenk.*, **13**, pp. 177-182.
- PAVLOVSKY, E. N. (1930). *Parasitology*, **22**, pp. 355-360.
- PAVLOVSKY, E. N. (1941). *C. R. Acad. Sci. U. R. S. S., (N. S.)* **31**, pp. 408-410.
- POSPELOVA-SHTROM, M. V. (1941). *Trav. Acad. milit. Méd.*, **25**, pp. 145-152.
- RAO, K. N. A. & KALRA, S. L. (1949). *Indian J. med. Res.*, **37**, pp. 385-394.
- ROUBAUD, E. & COLAS-BELCOUR, J. (1931). *Bull. Soc. Path. exot.*, **24**, pp. 948-957.
- SAPRE, S. N. (1944). *Indian J. vet. Sci.*, **14**, pp. 54-55.
- SAUTET, J. & WITKOWSKI, M. (1944). *Bull. Soc. Path. exot.*, **37**, pp. 182-188.
- STAROBYNSKY, A. (1922). *Presse méd.*, **30**, pp. 1445-1446.
- STELLA, E. (1940). *Riv. Biol. colon.*, **3**, pp. 431-435.
- TONELLI-RONDELLI, M. (1930). *Boll. Zool.*, **1**, pp. 113-115.
- VELU, H. (1919). *Bull. Soc. Path. exot.*, **12**, p. 99.
- WALTON, G. A. (1951). *E. Afr. med. J.*, **28**, p. 189.
- WARBURTON, C. (1918). *Parasitology*, **10**, pp. 284-285.
- WARBURTON, C. (1926). *Parasitology*, **18**, pp. 55-56.
- WARBURTON, C. (1933). *Parasitology*, **24**, pp. 558-568.
- WHITTICK, R. J. (1938). *Parasitology*, **30**, pp. 333-338.

THE EFFECT OF THE AGE AND STAGE OF DEVELOPMENT OF INSECT EGGS ON THEIR RESISTANCE TO INSECTICIDES.*

By E. H. SALKELD and C. POTTER.

*Department of Insecticides and Fungicides, Rothamsted Experimental Station,
Harpenden, Herts.*

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Only a little information is available on the relative resistance to the action of insecticides of the egg and other stages in the life-cycle of any given species of insect, but it is often supposed that the egg is the most resistant stage. Furthermore, it has been demonstrated that the age of the egg may have a direct bearing on its susceptibility to most insecticides. The differences in susceptibility which may be found to occur during the development of an insect egg may be caused by one of at least two factors or a combination of them. These factors are:—

- (1) Changes in the inherent susceptibility of the embryonic material.
 - (2) Changes in the permeability of the egg-shell and embryonic membranes.
- In addition, such factors as the absorption of the extra-embryonic fluids and

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the ingestion of the embryonic membranes which may contain accumulated insecticides must also be considered. However, since most insecticidal solutions must come in contact with and penetrate through the shell layers before they can exert a toxic action on the embryo, a detailed study of the structure and chemistry of the shell layers would appear to be of fundamental importance.

In this work a fairly detailed examination of the structure and permeability of the egg-shell and related membranes in the egg of the tomato moth, *Diataraxia oleracea* (L.) has been carried out. This has been made to determine if changes which might affect the entrance of insecticides to the embryonic material take place and, if such changes take place, the age of the egg at which they occur.

Spraying experiments using the insecticides DDT, HETP, allethrin and the triethanolamine salt of 3:5 dinitrocresol against eggs of *D. oleracea* of different ages were carried out to determine if differences in the resistance of the egg with age do occur and the extent of the differences when present. Finally, an attempt has been made to correlate differences in the toxicity of the insecticides to eggs of different ages with the stage of development of the embryo and with the structure and permeability of the protective envelopes at these particular ages. Any such correlation is complicated, since, in addition to the two factors of penetration and embryonic development which cannot easily be examined separately, other factors not yet recognised may be operating to affect the susceptibility of the egg.

Spraying experiments similar to those outlined above have been carried out using some of the same insecticides against eggs of different ages of the Mediterranean flour moth, *Ephestia kühniella* Zell. and of the cotton stainer, *Dysdercus fasciatus* Sign., to determine the resistance/age relationship in eggs of these species. This was done in order to determine if the resistance/age relationship varies from one insect order to another, and from one insect to another within the same order.

Review of Literature.

Much evidence has accumulated in the literature to indicate that the resistance of an insect egg to a toxic material may vary with the age of the egg. Many different chemicals have been tested against the eggs of many different species of insects, but in this review it is proposed to mention only a few of those tests in which a chemical in a liquid medium was applied to the egg as a spray or by a dipping technique (as a contact insecticide). This will exclude the very extensive work on the effect of fumigants on insect eggs.

The experiments considered here are those which give some information on the effect of the age of an insect egg on its susceptibility to the toxic substances used in this present work as well as those which give some information on the effect of these poisons on embryological development. In addition, experiments which deal with the structure and permeability of insect egg-shells as related to their protective function throughout the life of the egg have also been considered. It should be noted here that in many of these tests no definite age of egg was mentioned as being either the most or the least susceptible, the reference to the age of the egg being made as either the youngest or the oldest egg tested. However, a review of these tests will serve to indicate that, in general, the age of an insect egg has an important bearing on its resistance to an insecticide and also that the concentration, formulation and method of application of the insecticide are important considerations when determining differences in resistance of various ages of eggs.

Results obtained in such tests have led to many speculative theories as to the mode of action of ovicides, but only fairly recently have attempts been made to

correlate these changes in susceptibility with changes in the structure and permeability of the chorion and embryonic membranes of eggs of different ages and to a lesser extent with the various physiological and morphological changes in the living embryological material.

Although no reference to the effect of allethrin as an ovicide was found in the literature, considerable information is available on the ovicidal action of the pyrethrins. Since allethrin is closely related chemically to the insecticidal constituents of pyrethrum it seems possible that their ovicidal action may be similar. Onoe and Fukuda (1939) found in laboratory experiments that pyrethrum extracts in a 0.5 per cent. soap solution were more effective against one-week-old eggs of the oriental rice borer, *Chilo simplex* (Butler), than they were against one-day-old eggs. Similarly, Mori (1940) found the young eggs of this species of insect to be more resistant to extracts of pyrethrum in acetone than the older eggs. Maercks (1935) used a dipping method to test the ovicidal effect of an aqueous solution of pyrethrum on the eggs of both *Carpocapsa pomonella* (L.), and *Polychrosis botrana* (Schiff.). By using the eggs at 5 different stages of development, he showed that the older eggs were more susceptible than the younger eggs. Potter and Tattersfield (1943) carried out laboratory spraying and dipping experiments with pyrethrum in an acetone-sulphonated lorol-water medium on the eggs of *Pieris brassicae* (L.), *Ephestia kühniella*, *Sitotroga cerealella* (Ol.), and *Plutella maculipennis* (Curt.). They noticed that a high proportion of the unhatched eggs contained embryos that were either fully developed or highly developed before dying, but that some eggs were killed with partially developed embryos indicating that a toxic action was being exerted before full development had been attained. Mukerjee (1953) determined that the susceptibility of the eggs of *Diataraxia oleracea* to pyrethrins in an acetone-sulphonated lorol-water medium varied with the age of the eggs. He found that this susceptibility increased rather sharply from the 1-day (youngest egg) to the 2-day-old egg and then gradually increased to the 5-day-egg (oldest egg) which was the most susceptible. He also noticed that the pyrethrins had an effect on the development of the embryo and that in some cases further embryonic development was inhibited after the application of the insecticide.

A few experiments in which DDT was tested as an ovicide have been reported and it appears that, in some cases, no differences in susceptibility with the age of the egg were noticed. It has been pointed out by Speyer and Parr (1945) that they found no differences in the susceptibility of various ages of eggs of *D. oleracea* when they were dusted with a 5 per cent. DDT powder or when sprayed with a DDT emulsion. When sprays containing DDT in emulsion at concentrations ranging from 0.013 per cent. to 0.05 per cent. were applied to egg-batches at periods of from 4 to 9 days after their deposition, none of the larvae hatched although they developed fully, and it was concluded that the DDT penetrated the egg-shell in quantities insufficient to prevent development of the larva but sufficient to enfeeble it to a degree which prevented it from perforating the shell. Also Ludwig (1946) found that when the eggs of *Popillia japonica* Newm. were exposed to DDT on successive days of their embryonic development by placing them for 10 minutes on a filter paper moistened with either a 5 per cent. or a 10 per cent. solution of DDT, DDT had no effect on the embryonic development. No significant difference in susceptibility of the eggs with age was noticed although high mortalities were recorded for the newly laid eggs. This might have been caused, according to Ludwig, by heavy growths of moulds which occurred. Again Mukerjee (1953) tested eggs of four ages of *Diataraxia oleracea* with a suspension of DDT in an acetone-sulphonated lorol-water medium. He found that the insecticide did not stop the development of the larvae in eggs of any

age but that the oldest egg tested was the most susceptible. These susceptibility differences were not very significant statistically.

The dinitro compounds have long been recognised as potent ovicides (Tattersfield & others, 1925; Gimingham & others, 1926). Gimingham and Tattersfield (1927) when testing the toxicity of 3:5 dinitro-o-cresol in a benzene-saponin-water medium and the sodium salt of 3:5 dinitro-o-cresol in a saponin-water medium to eggs of several species of moth under uncontrolled conditions of temperature and humidity found that hatching was not entirely prevented in eggs of some species by the action of low concentrations of the poison. They obtained some evidence that the eggs of various insects were more resistant to the toxic action of dinitrocresol when they were at an advanced stage of development. Batches of eggs of different ages of *Tortrix pronubana* Hb. were treated (sprayed) with three concentrations of dinitro-o-cresol (0.1 per cent., 0.05 per cent. and 0.025 per cent.) and in all cases 100 per cent. mortality occurred. As a result, no differences in resistance with age were noted. Observations on the stage of development of the embryo reached at the time of spraying showed that little further development took place and they suggested, therefore, that the penetration and toxic action of the poison was very rapid.

Potter and Tattersfield (1943) also observed that when 3:5 dinitro-o-cresol in an acetone-sulphonated loral-water medium was sprayed on eggs of *Ephestia kühniella* (varying in age from 0-2 days) further development of the embryo was inhibited in many of the eggs killed. Chancogne and others (1949) and Gaumont (1951) in laboratory tests with sodium dinitrocresylate against different ages of the egg of *Operophtera brumata* (L.) both found that the age of the egg had an effect on its susceptibility to the poison. Susceptibility was greater immediately after the egg was laid (youngest eggs) and before the larva hatched (oldest eggs) than at any other stage of embryonic development. However, Dierick (1942) when testing various preparations of dinitro-o-cresol against eggs of different ages of *Ephestia kühniella* observed that his substance was more effective in killing the older eggs (5-6 days old) than the younger eggs (0-1 day old).

Much experimental work, both in laboratory and field tests, has been carried out on the ovicidal effects of 3:5 DNOC and other dinitrocresylates when combined with petroleum oils and used as winter washes. These tests will not be reviewed in this work because it would appear to be very difficult to separate the action of the DNOC from that of the oils, and as has been mentioned previously only the relevant literature on the poisons used in this work is being considered.

Neither HETP- nor TEPP-containing materials appear to have been used to any extent as ovicides, and those tests which have been made seem to indicate that these materials are usually ineffective. Petty (1948) found that HETP used as a spray was not effective in killing the eggs of *Tetranychus bimaculatus* Harvey, and Zimmerman and Hartzell (1947) also found that the eggs of the same species and of *Pseudococcus citri* (Risso) were not killed in fumigation tests with HETP and TEPP in greenhouses. However, Lord and Potter (1951) have studied the contact ovicidal effect of a TEPP-containing material on eggs of *Diataraxia oleracea* and *Ephestia kühniella* and have found that the sample used was toxic to the eggs of both species although high concentrations were required to kill. They further noted that at a low dosage a high percentage of the eggs formed highly developed embryos before death but that at higher dosages the development of the embryo was inhibited even in the young eggs in which no visible embryonic development had taken place.

It appears from the literature reviewed here that high concentrations of the pyrethrins, HETP and some of the dinitro compounds can inhibit the development of the embryo in eggs of any age. DDT, however, appears to be incapable

of exerting this action. The fact that an egg containing only undifferentiated yolk cells can be killed by such poisons as pyrethrins and HETP which are generally believed to act on some part of the nervous system (see Metcalf, 1948) is of interest.

It also appears that the susceptibility to these poisons of eggs of many species of insect varies with the age of the eggs. Although in some of these experiments such changes in susceptibility have been correlated with changes in the embryonic development of the egg, no account has been taken of the effect which changes that might occur in the egg-shell may have on the penetration of the poison.

It seems logical to suppose that the resistance of an insect egg to a toxic material may depend not only on the susceptibility of the embryonic material but also on the ability of the toxic material to penetrate through the shell layers to the embryo. Experiments on the penetration of some types of oil through the shells of several species of insect eggs have been done by Fox (1930), O'Kane and Baker (1934, 1935) and Blickle (1942). O'Kane and Baker (1935) dipped eggs of the Gryllid, *Oecanthus niveus* (Deg.), the Coccinellid, *Epilachna corrupta* Muls., the Coreid, *Anasa tristis* (Deg.), the Aphid, *Dilachnus pini* (L.), the lacewing, *Chrysopa oculata* Say, and the moth, *Samia cecropia* (L.), in oil for a given length of time and then sectioned and stained the eggs. They found traces of oil in the chorion, in the layers underlying the chorion and surrounding the yolk and in the developing embryo in the eggs of all these insect species. Blickle using pine oil saturated in a dye found that the oil (as judged by the presence of the dye) penetrated the shell of the older eggs of *Epilachna corrupta* more quickly than the younger eggs. This indicated that some change was taking place in the protective envelopes of the egg as hatching time approached.

Recently more interest has been shown in attempts to determine the structure and chemical nature of insect egg-shells and some correlation between structural and developmental changes in the egg-shell with changes in the susceptibility of the egg to toxic substances has been made. Much work has been done to determine the nature and permeability of the egg membranes of the Acridid, *Melanoplus differentialis* (Thos.) by Jahn (1935a, b) and by Slifer (1930, 1937, 1938, 1948, 1949a). Slifer (1949b) was able to demonstrate that at two periods during the development of the eggs of *M. differentialis* the eggs are more susceptible to the action of an aqueous solution of iodine while at other times they are highly resistant and she points out that this fact might be of considerable interest to applied entomologists. She has correlated these changes in resistance with the presence or absence of an impermeable wax layer in the chorion—the egg being more resistant when the complete wax layer is present. Beament (1946a) has carried out an extensive study on the formation and structure of the chorion of the egg of the Reduviid, *Rhodnius prolixus* Stål. He has shown that this structure is very complex, and that there are, in this egg-shell, seven layers of proteinaceous material which in themselves are not impermeable to water. However, he has established (Beament, 1946b) that, in addition to the proteinaceous layers, there is an initial waterproofing mechanism in the egg consisting of a very thin layer of wax (the primary wax layer) which covers the inside of the chorion and which is present when the egg is laid. As the egg develops, membranes are added to the inner surface of the chorion by the oöcyte and at a certain stage in the development of the egg these embryonic membranes become impregnated with a high-melting-point wax which further waterproofs the eggs (Beament, 1949). By carrying out studies on the penetration of various liquids through the protective envelopes (chorion and sub-chorial) of eggs of different ages of *R. prolixus*, Beament (1948, 1949) has shown that considerable differences exist in the ability of both hydrophilic and lipophilic fluids to enter the egg. He found that a very close correlation existed between these changes in the

resistance of the egg to penetration and the changes found in the protective envelopes of the egg. In general, the resistance to hydrophiles increased during development due to the formation of the embryonic membranes and their subsequent impregnation with a high-melting-point wax and then decreased shortly before hatching when these membranes were broken down by the digestive enzymes of the embryo. Resistance to lipophiles increased during the formation of the embryonic membranes and then decreased slightly when the wax impregnation of this membrane took place and, of course, decreased still further as this membrane was broken down before hatching.

Davies (1948) has carried out a study on the egg-shell of the Calliphorid, *Lucilia sericata* (Mg.) to determine the effect of humidity on egg development and hatching. He found that a waterproofing layer was laid down by the oöcyte between the chorion and the chorionic vitelline membrane. A somewhat similar wax layer has been found in most insect eggs studied: e.g., in *Rhodnius prolixus* (Beament, 1946a), in *Melanoplus differentialis* (Slifer, 1948), and in *Locustana pardalina* (Wlk.) (Matthée, 1951). In tick eggs, however, the means by which the shell is waterproofed appears to be slightly different. Lees and Beament (1948) have shown that the resistance to desiccation of the eggs of the ticks, *Ixodes ricinus* (L.) and *Ornithodoros moubata* (Murr.) was due to an outer coating of wax which covered the egg-shell. Beament (1951) has found that the eggs of *Metatetranychus ulmi* (Koch) (Acarina) are covered externally with a thick layer of wax but also have a thin wax layer on the inner surface of the chorion similar to the wax layer in most insect eggs.

Very little information concerning the structure or permeability of the egg membranes in Lepidoptera could be found. Leuckart (1855) examined the chorion and micropylar structure of eggs in several orders of insects, and many Lepidopterous eggs were included in this work. Korschelt (1884, 1887) described the formation of the chorion in several Lepidoptera and Müller (1938) has done similar work with *Plodia interpunctella* (Hb.). Gross and Howland (1940) described the micropylar structure of *Prodenia eridania* (Cram.) in their paper on the early embryology of this species. However, most of these descriptions dealt only with the morphological characteristics and very little attempt was made to determine the physiological function of the shell and its related membranes. However, Wigglesworth and Beament (1950) have studied the respiratory mechanism in the eggs of several insect species including *Bombyx mori* (L.), and *Ephestia kühniella* and in so doing have described briefly the gross structure of these shells.

Materials and Methods.

The apparatus used in all the spraying experiments has been described by Potter (1952). The methods of preparing the eggs for spraying, the actual spraying, the incubation of the eggs after treatment and the assessment of effect were standardised for each group of experiments with eggs of one species of insect.

Biological.

Eggs of the following three species of insects were used for the spraying tests.

(1) Tomato moth, *Diataraxia oleracea* (L.).

The methods of raising this insect in the laboratory have been described by Way and others (1951), and of obtaining eggs of a known age by Mukerjee (1953). The length of time before hatching occurs depends upon the incubation temperature. By removing newly laid eggs from the laying cages each morning and incubating them at 75°F., five different ages were obtained for experimental purposes. Similarly, by incubating them at 57°F. fourteen ages were obtained.

Because it was not feasible to carry out spraying experiments with fourteen ages of eggs, five ages were chosen in which the development of the embryos corresponded as closely as possible to the embryonic development in the five ages of eggs obtained at 75°F. Differences in humidity did not appear to affect either the length of the incubation period or the natural mortality of the eggs (see also Way & others, 1951).

Eggs from any one age group were removed from the muslin or paper on which they had been laid and were thoroughly randomised. Then 15 to 20 eggs from each age group were placed within circles marked on a piece of tricoline cloth which fitted into the bottom of a petri dish. Each circle was labelled with the age of the eggs it contained. In this way representative eggs from each of the five age groups were collected in one dish and the spraying treatment was, therefore, the same for all. Three replicates were carried out for each concentration of poison. A paint brush moistened in water was used to separate the eggs from the batches in which they were laid and to transfer them to the tricoline. The cement on the chorion of the eggs was softened by the water but became hardened again as the water evaporated and held the eggs firmly in position on the tricoline substrate. The eggs were prepared in this way at least five hours before they were sprayed to allow the water to evaporate because it has been pointed out by Beament (1948) that the presence of water on the chorion of an insect egg may affect the action of both lipophilic and hydrophilic insecticides.

The eggs were incubated at the same temperature and humidity both before and after spraying unless otherwise stated.

(2) Mediterranean flour moth, *Ephestia kühniella* Zell.

The method of rearing this insect and of obtaining the eggs was much the same as that used by Potter and Tattersfield (1943). Five different ages of eggs for test purposes were obtained by using an incubation temperature of 75°F. The relative humidity varied between 60 per cent. and 70 per cent.

The method of handling these eggs during spraying was almost identical with that used for the eggs of *D. oleracea*. Only a very slightly moistened paint brush was required for moving these eggs to the tricoline substrate.

(3) Cotton stainer, *Dysdercus fasciatus* Sign.

Approximately 25 pairs of egg-laying adults of *D. fasciatus* were placed in a small pail (7 lb. honey pail) the bottom of which was covered with a filter paper. Because the eggs would not survive without a high humidity, a petri dish containing moist cotton wool was placed in the bottom of the pail. Most of the females laid their eggs under the edge of this wool. The top of the pail was covered with muslin held in place by a rubber band. Food, which consisted of a handful of cotton seed, was placed on top of the muslin and another piece of damp cotton wool was put on top of the seeds to keep them moist. The petri dish containing the eggs was removed each morning and the eggs were incubated in a saturated atmosphere at 75°F. Under these conditions of temperature and humidity hatching took place in 8 days.

In the spraying tests, it was found necessary to confine the eggs for each age group to be tested within a shallow glass ring (height $\frac{1}{4}$ in., width 1 in.) as a method of preventing them from rolling about on the substrate. Three such circles fitted into one petri dish. The actual spraying procedure was the same as for *Diataraxia oleracea* and *E. kühniella*, but after treatment the eggs were kept at a high humidity.

Assessment of effect.

The treated eggs were inspected as soon as the control eggs, *i.e.*, those that had been sprayed with the medium only, had hatched. Eggs regarded as hatched were those from which the larvae had completely freed themselves from the chorion. In the case of *Dysdercus fasciatus*, a hatched egg was one from which the nymph had emerged completely.

Unhatched eggs were examined to determine the stage of embryonic development reached at the time death occurred.

Analysis of the data was made by the standard method of probits and dosages (Finney, 1947). The median lethal concentration (M.L.C.) of each insecticide for each age group (the concentration of insecticide required to prevent hatching in 50 per cent. of the eggs) was obtained from the regression equation. The relative resistance of each age group was calculated from the M.L.C. by assigning an arbitrary value of 1.00 as the resistance of the oldest age group. Two or more experiments were carried out with each insecticide against eggs of each species of insect. When using any one insecticide, the median lethal concentrations found for each age group were averaged and the average relative resistance for each age of egg was obtained from these figures. When the difference between the relative resistances of two age groups was small the figures were analysed for significance (Finney, 1947).

The slope of the probit lines obtained for the different ages of eggs varied considerably. In addition, the slopes of the probit lines obtained for one age of eggs in two separate experiments with the same insecticide were not the same although theoretically they should have been parallel. Since the relative resistances in these experiments have been obtained from the M.L.C.s, it should be realised that the trend of a relative resistance/age curve obtained from either the LD75 or the LD25 might not be the same as that found for the LD50.

Chemical.

Various experiments were carried out in which some or all of the following insecticides were used:—

- (1) DDT.—pure crystallised 2,2,bis (parachlorophenyl) 1,1,1, trichloroethane applied as a suspension of unknown crystal size. Its melting point was 107.5–108°C.
- (2) The triethanolamine salt of 3:5 dinitro-ortho-cresol (water-soluble). Throughout this paper this insecticide will be referred to as TDNOC.
- (3) Allethrin—an approximately 100 per cent. pure sample of the synthetic allyl homologue of cinerin I.
- (4) HETP.—a sample of hexaethyl tetraphosphate containing approximately 30 per cent. tetraethyl pyrophosphate.

All the insecticidal solutions were prepared in an aqueous medium containing either 5 per cent. benzene in 0.1 per cent. Lissapol N or 10 per cent. acetone in 0.1 per cent. sulphonated lorol except the triethanolamine salt of 3:5 dinitro-ortho-cresol which was water soluble and was used in a medium of 0.1 per cent. sulphonated lorol.

Spraying Experiments.

I. Diataraxia oleracea.

(a) DDT. Three separate spraying tests (i, ii, iii) were carried out, the experimental details and results of which are given in Table I.

The median lethal concentration obtained for each age of egg varied slightly from experiment to experiment but the overall trend in the magnitude of the M.L.C.s from one age group to another remained much the same.

Fig. 1 depicts the relative resistance to DDT of each age group used in the three experiments and the average relative resistance calculated for each age. It can be seen under the conditions of test (75°F. both before and after treatment) the relative resistance appears to decrease very slightly from the youngest to the oldest egg, the youngest egg being about one and a half times as resistant as the oldest, but this difference was not statistically significant.

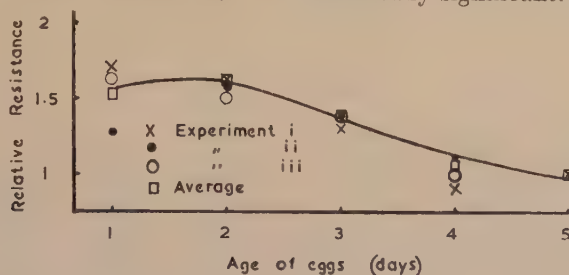


Fig. 1.—DDT. The relative resistances of five ages of eggs of *D. oleracea* (three separate experiments) at 75°F. and 66%–70% R.H., and the calculated average value for each age of egg.

In all eggs sprayed with DDT, the embryonic development proceeded normally and death only occurred when the larvae attempted to hatch by eating their way out of the shell. Mukerjea (1953) observed similar results with DDT against eggs of *D. oleracea*, as did Ludwig (1946) when using DDT against eggs of *Popillia japonica*.

A very high concentration (5 per cent.) of DDT did not inhibit the embryonic development in any of the five ages of eggs tested until hatching time. In a small percentage of the eggs, especially the younger ages, the larvae developed normally but made no apparent attempt to hatch. Weak larval movements could be seen in these eggs several hours after the control eggs had hatched. Finally the larvae became desiccated and shrivelled to the floor of the chorion. A test against one-day-old eggs showed that 80 per cent. of the larvae developed normally and attempted to hatch and that 20 per cent. developed normally (apparently) but did not attempt to hatch.

Concentrations of DDT sufficient to cause 100 per cent. mortality but not sufficient to prevent attempts at hatching did not appear to delay the development of the embryos because both DDT-sprayed and medium-sprayed eggs began hatching at about the same time.

(b) The triethanolamine salt of 3:5 dinitro-ortho-cresol. Two spraying experiments (i and ii) were carried out against five ages of eggs using an aqueous solution of 3:5 TDNOC at 75°F. and at a relative humidity of 66–70 per cent. It was found that there was very little difference in the relative resistances of the eggs of different ages under these conditions (see fig. 2, experiments i and ii). There was only a slight indication that resistance decreased shortly before hatching took place. Because it has been reported by Viel and Chancogne (1951) that sodium dinitrocresylate was more toxic at high humidities to the eggs of *Ephestia kühniella* it was thought of interest to determine if a high humidity would alter the resistance/age relationship in the eggs of *D. oleracea*. Two experiments were set up (iii and iv) in which the eggs in experiment iii were kept at 75°F. and 60 per cent. R.H. and the eggs in experiment iv at 75°F. and 90 per cent. R.H. both before and after spraying.

TABLE I.

D. oleracea. The effect of the age of eggs on their susceptibility to DDT. Details of three spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration $\times 10^3$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y = 3.0413x + 0.8154$	1.375 ± 0.069	0.024	3.0413 ± 0.69	1.71	1.52
	(ii) $Y = 1.9215x + 2.3956$	1.354 ± 0.071	0.023	1.9215 ± 0.40	1.28	
	(iii) $Y = 5.0495x + 1.8666$	1.360 ± 0.025	0.023	5.0495 ± 0.78	1.64	
2	(i) $Y = 2.2610x + 1.9323$	1.358 ± 0.071	0.023	2.2610 ± 0.41	1.64	1.57
	(ii) $Y = 4.2775x - 1.1893$	1.446 ± 0.031	0.028	4.2775 ± 0.58	1.56	
	(iii) $Y = 5.1461x + 1.8007$	1.320 ± 0.023	0.021	5.1461 ± 0.69	1.50	
3	(i) $Y = 2.9186x + 1.3218$	1.260 ± 0.075	0.018	2.9186 ± 0.58	1.29	1.35
	(ii) $Y = 4.3626x - 1.1073$	1.401 ± 0.031	0.025	4.3626 ± 0.60	1.39	
	(iii) $Y = 5.7869x + 2.4538$	1.287 ± 0.026	0.019	5.7869 ± 1.05	1.36	
4	(i) $Y = 3.1266x + 1.5332$	1.109 ± 0.082	0.013	3.1266 ± 0.80	0.93	1.02
	(ii) $Y = 3.8312x - 0.0128$	1.303 ± 0.045	0.020	3.8312 ± 0.66	1.11	
	(iii) $Y = 6.4452x + 2.4183$	1.150 ± 0.036	0.014	6.4452 ± 1.51	1.00	
5	(i) $Y = 2.5134x + 2.1039$	1.155 ± 0.068	0.014	2.5134 ± 0.60	1.00	1.00
	(ii) $Y = 4.0552x - 0.0872$	1.254 ± 0.039	0.018	4.0552 ± 0.58	1.00	
	(iii) $Y = 4.0065x + 0.4418$	1.137 ± 0.043	0.014	4.0065 ± 0.71	1.00	

Dates of spraying: (i) 20/10/50, (ii) 1/11/50, (iii) 8/11/50.

Medium: an aqueous medium containing 5% benzene and 0.1% Lissapol N.

Total number of eggs used per concentration: 60 (approx).

Spray deposit: 7.7-9.1 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after treatment); humidity, 66%-70%.

In comparing the results of these two experiments (see Table II, experiments iii and iv) it seems apparent that a lower humidity decreases the toxicity of TDNOC to eggs of *D. oleracea*, especially to the 1-, 2-, 3- and 4-day-old eggs.

Because the trends of the relative resistances are much the same in these two experiments and are also much the same as those found in experiments i and ii, the data from all four experiments have been included in Table II and have been used in the determination of the average relative resistance in fig. 2.

The age of the egg did not appear to have any great effect on its resistance to TDNOC when the insecticide was applied under the experimental conditions

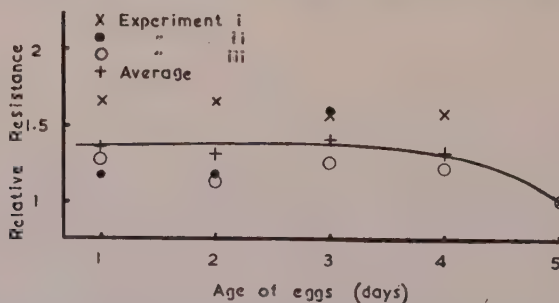


Fig. 2.—TDNOC. The relative resistances of five ages of eggs of *D. oleracea* (four separate experiments) and with the calculated value for each age of egg. Experiments i and ii were carried out at 75°F. and 66%-70% R.H., experiment iii at 75°F. and 60% R.H., and experiment iv at 75°F. and 90% R.H.

TABLE II.

D. oleracea. The effect of the age of eggs on their susceptibility to the triethanolamine salt of 3:5 dinitro-ortho-cresol. Details of four spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration (i) $\times 10^3$ (ii-iv) $\times 10^2$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y=4.9770x - 8.4365$	2.699 ± 0.033	0.500	4.9770 ± 0.96	1.17	1.10
	(ii) $Y=4.9617x - 2.5700$	1.526 ± 0.031	0.336	4.9617 ± 0.68	0.93	
	(iii) $Y=4.9284x - 2.3803$	1.497 ± 0.031	0.316	4.9284 ± 0.55	1.37	
	(iv) $Y=4.2708x - 0.5930$	1.309 ± 0.032	0.204	4.2708 ± 0.56	0.93	
2	(i) $Y=2.5905x - 2.0960$	2.741 ± 0.071	0.551	2.5905 ± 1.02	1.29	1.13
	(ii) $Y=2.7516x + 1.1368$	1.404 ± 0.062	0.254	2.7516 ± 0.49	0.71	
	(iii) $Y=4.7000x - 2.2843$	1.549 ± 0.036	0.354	4.7000 ± 0.71	1.54	
	(iv) $Y=5.0819x - 1.9665$	1.372 ± 0.041	0.236	5.0819 ± 0.82	1.07	
3	(i) $Y=2.6489x - 2.3103$	2.758 ± 0.054	0.573	2.6489 ± 0.87	1.34	1.20
	(ii) $Y=3.2990x - 0.2386$	1.588 ± 0.094	0.387	3.2990 ± 0.38	1.08	
	(iii) $Y=5.5640x - 3.5285$	1.534 ± 0.031	0.342	5.5640 ± 0.83	1.49	
	(iv) $Y=4.1264x - 0.2031$	1.259 ± 0.040	0.182	4.1264 ± 0.63	0.83	
4	(i) $Y=5.3746x - 9.7352$	2.745 ± 0.034	0.556	5.3746 ± 1.23	1.30	1.25
	(ii) $Y=3.1341x - 0.0217$	1.604 ± 0.042	0.402	3.1341 ± 0.49	1.12	
	(iii) $Y=4.6356x - 2.1963$	1.552 ± 0.031	0.357	4.6356 ± 0.62	1.55	
	(iv) $Y=2.7646x - 1.2549$	1.359 ± 0.044	0.229	2.7646 ± 0.35	1.04	
5	(i) $Y=3.2816x - 3.6255$	2.631 ± 0.051	0.428	3.2816 ± 0.85	1.00	1.00
	(ii) $Y=2.3881x + 1.2806$	1.556 ± 0.058	0.360	2.3881 ± 0.48	1.00	
	(iii) $Y=2.3167x + 1.8428$	1.362 ± 0.075	0.230	2.3167 ± 0.46	1.00	
	(iv) $Y=3.2216x + 0.6764$	1.342 ± 0.036	0.220	3.2216 ± 0.32	1.00	

Dates of spraying: (i) 8/3/51, (ii) 20/11/51, (iii) 30/11/51, (iv) 30/11/51.

Medium: an aqueous medium containing 0.1% sulphonated lorol.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 7.2-9.3 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after treatment); humidity, (i) and (ii) 66%-70%, (iii) 60%, (iv) 90%.

described. The 5-day-old eggs were the least resistant but this difference was barely significant statistically.

In an attempt to determine if larger differences in the relative resistances of eggs of *D. oleracea* could be found, the incubation period of the eggs was lengthened to 14 days by decreasing the incubation temperature before and after spraying to 57°F. Three experiments were carried out using eggs of ages 1 day, 4 days, 7 days, 10 days and 14 days. The experimental details and results are given in Table III and the relative resistances found for each age of egg together with the average relative resistance are given in fig. 3. These figures indicate that there is a considerable increase in resistance from the 1-day to the 4-day egg. This resistance increases slightly to the 7-day egg and then gradually decreases to the 10-day egg followed by a sharp decrease to the 14-day egg immediately before hatching occurs. A somewhat similar result was obtained by Hough (1939) when he found that sodium dinitro-o-cresolate was more effective when used just prior to the hatching of the eggs of the apple aphids than when applied earlier.

A comparison of the average M.L.C. for any one age of egg at 75°F. with that found for an egg having a similar embryological development at 57°F. shows

TABLE III.

D. oleracea. The effect of the age of eggs on their susceptibility to the triethanolamine salt of 3:5 dinitro-ortho-cresol. Details of three spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration (i) & (ii) $\times 10^2$ (iii) $\times 10^3$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y = 6.8102x - 3.9291$ (ii) $Y = 1.6441x + 2.6953$ (iii) $Y = 2.4799x - 0.7622$	1.311 ± 0.024 1.402 ± 0.054 2.323 ± 0.086	0.205 0.252 0.210	6.8102 ± 1.06 1.6441 ± 0.22 2.4799 ± 0.42	1.21 2.05 1.63	1.58
4	(i) $Y = 5.5173x - 2.6844$ (ii) $Y = 2.0566x + 1.7350$ (iii) $Y = 3.4853x - 3.7553$	1.391 ± 0.023 1.583 ± 0.068 2.510 ± 0.075	0.246 0.383 0.324	5.5173 ± 0.92 2.0566 ± 0.49 3.4853 ± 0.74	1.46 3.11 2.51	2.26
7	(i) $Y = 5.7294x - 4.0199$ (ii) $Y = 1.7151x + 2.2411$ (iii) $Y = 4.4502x - 7.2099$	1.574 ± 0.016 1.605 ± 0.054 2.744 ± 0.039	0.375 0.403 0.555	5.7294 ± 0.68 1.7151 ± 0.32 4.4502 ± 0.82	2.22 3.28 4.30	3.17
10	(i) $Y = 8.3689x - 7.9350$ (ii) $Y = 1.6363x + 2.5258$ (iii) $Y = 4.2647x - 6.2699$	1.546 ± 0.011 1.506 ± 0.055 2.646 ± 0.053	0.352 0.321 0.443	8.3689 ± 0.91 1.6362 ± 0.27 4.2647 ± 0.84	2.08 2.61 3.43	2.65
14	(i) $Y = 5.9550x - 2.3179$ (ii) $Y = 1.4613x + 3.4101$ (iii) $Y = 2.2901x + 0.1711$	1.228 ± 0.022 1.089 ± 0.060 2.109 ± 0.11	0.169 0.123 0.129	5.9550 ± 1.51 1.4613 ± 0.23 2.2901 ± 0.45	1.00 1.00 1.00	1.00

Dates of spraying: (i) 8/12/51, (ii) 6/12/51, (iii) 4/11/51.

Medium: an aqueous medium containing 0.1% sulphonated loral.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 7.0-9.6 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 57°F. (before and after spraying); humidity, 60%-70%.

that lower concentrations are required at 57°F. to cause a 50 per cent. kill in the youngest and oldest eggs. The LD50's for the other ages of eggs are much the same regardless of the incubation temperature.

Observations on the stage of development which the embryo had reached at the time death occurred showed that fairly high concentrations of TDNOC (1.0 per cent. to 2.0 per cent. at 75°F.) were able to stop the development of the

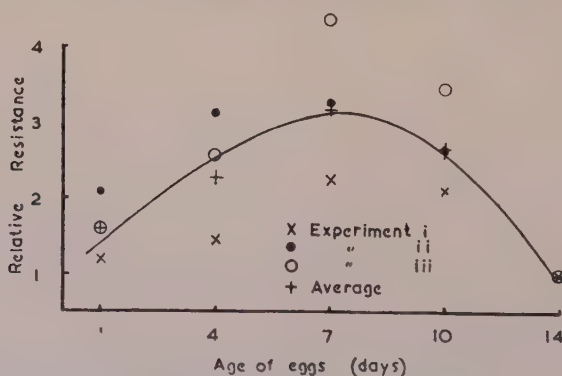


Fig. 3.—TDNOC. The relative resistance of five ages of eggs of *D. oleracea* (three separate experiments) at 57°F. and 60%-70% R.H., and the calculated average value for each age of egg.

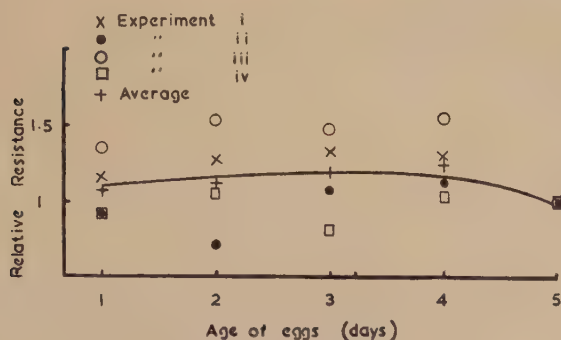


Fig. 4.—Allethrin. The relative resistances of five ages of eggs of *D. oleracea* (three separate experiments) at 75°F. and 60%–70% R.H., and the calculated relative value for each age of egg.

embryo almost immediately they were applied to the egg. The lower the concentration used the higher the number of embryos which became fully developed. At very low concentrations (0.1 per cent.) the death of the embryo only occurred as it began to hatch. It was noted that very young eggs of *D. oleracea* in which the development of the embryo had been stopped by the TDNOC turned brown in colour and several small brown circles which appeared to be composed of yolk material became closely applied to the chorion. Gimingham and others (1926)

TABLE IV.

D. oleracea. The effect of the age of eggs on their susceptibility to allethrin. Details of three spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration $\times 10^3$	Median lethal concentration	Slope of probit line (b)	Relative re-sistance	Average relative re-sistance
1	(i) $Y = 4.3784x - 4.1715$	2.094 ± 0.042	0.124	4.3784 ± 0.81	1.68	1.37
	(ii) $Y = 4.3306x - 3.3878$	1.938 ± 0.027	0.087	4.3306 ± 0.55	1.18	
	(iii) $Y = 2.7076x + 2.1907$	1.036 ± 0.030	0.109	2.7076 ± 0.27	1.28	
2	(i) $Y = 8.7897x - 13.3557$	2.089 ± 0.024	0.123	8.7897 ± 0.41	1.67	1.32
	(ii) $Y = 3.8512x - 2.4741$	1.940 ± 0.036	0.087	3.8512 ± 0.50	1.18	
	(iii) $Y = 3.4726x + 1.5837$	0.986 ± 0.035	0.097	3.4726 ± 0.46	1.14	
3	(i) $Y = 2.5000x - 0.1640$	2.064 ± 0.064	0.116	2.5000 ± 0.16	1.58	1.43
	(ii) $Y = 3.6188x - 2.4110$	2.047 ± 0.032	0.111	3.6188 ± 0.49	1.51	
	(iii) $Y = 4.8100x + 0.0421$	1.031 ± 0.034	0.107	4.8100 ± 0.73	1.26	
4	(i) $Y = 5.7002x - 6.7936$	2.068 ± 0.027	0.117	5.7022 ± 0.66	1.59	1.32
	(ii) $Y = 4.0734x - 2.8517$	1.929 ± 0.033	0.085	4.0734 ± 0.79	1.15	
	(iii) $Y = 3.9203x + 0.9955$	1.020 ± 0.025	0.105	3.9203 ± 0.39	1.24	
5	(i) $Y = 4.0469x - 2.5597$	1.867 ± 0.038	0.074	4.0469 ± 0.70	1.00	1.00
	(ii) $Y = 5.8549x - 5.9153$	1.867 ± 0.025	0.074	5.8549 ± 0.55	1.00	
	(iii) $Y = 3.5971x + 1.6629$	0.928 ± 0.022	0.085	3.5971 ± 0.31	1.00	

Dates of spraying: (i) 5/10/50, (ii) 21/3/51, (iii) 21/11/51.

Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated lorol.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 9.0–9.5 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after spraying); humidity, 60%–70%.

and Gimingham and Tattersfield (1927) found a somewhat similar action for dinitro-cresol and its sodium salt on the eggs of *Tortrix pronubana* in an early stage of development but observed that this discoloration and disorganisation of the egg contents was less marked in the case of the more developed eggs.

The results of Chancogne and others (1949) and Gaumont (1951) on the susceptibility of different ages of the egg of *Operophtera brumata* to sodium nitro-cresylate appear to agree very closely with the resistance/age relationship of eggs of *D. oleracea* to 3:5 TDNOC at 57°F. as found in this present work. 141

(c) Allethrin. Three spraying tests were carried out at 75°F. and 60 per cent. to 70 per cent. R.H. with allethrin (experiments i, ii and iii). The experimental conditions and details together with the results are given in Table IV. The relative resistances and the average relative resistance for each age of egg are given in fig. 4. Very little differences in the susceptibility of the 1-, 2-, 3- and 4-day eggs were noticed in any of the three experiments but the 5-day egg was the most susceptible in all cases.

TABLE V.

D. oleracea. The effect of the age of eggs on their susceptibility to allethrin. Details of three spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration (i) & (iii) $\times 10^3$ (ii) $\times 10^2$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y = 1.9545x + 2.3160$	1.374 ± 0.071	0.0237	1.9545 ± 0.28	2.30	1.83
	(ii) $Y = 3.7229x - 0.2225$	1.403 ± 0.036	0.0253	3.7229 ± 0.47	2.24	
4	(i) $Y = 2.1126x + 1.6174$	1.602 ± 0.064	0.0400	2.1126 ± 0.29	3.88	3.07
	(ii) $Y = 4.5040x - 2.5775$	1.684 ± 0.033	0.0483	4.5040 ± 0.89	4.27	
	(iii) $Y = 3.1408x + 3.2828$	0.548 ± 0.037	0.0353	3.1408 ± 0.40	1.89	
7	(i) $Y = 2.7854x + 0.4911$	1.616 ± 0.039	0.0413	2.7854 ± 0.33	4.00	3.21
	(ii) $Y = 7.3283x - 7.3919$	1.690 ± 0.021	0.0490	7.3283 ± 1.10	4.34	
	(iii) $Y = 3.0447x + 3.2206$	0.586 ± 0.035	0.0386	3.0447 ± 0.36	2.06	
10	(i) $Y = 2.9197x + 0.7576$	1.452 ± 0.043	0.0283	2.9197 ± 0.39	2.75	2.05
	(ii) $Y = 3.6547x - 0.3650$	1.471 ± 0.037	0.0296	3.6547 ± 0.54	2.62	
	(iii) $Y = 3.5981x + 3.6023$	0.389 ± 0.058	0.0245	3.5981 ± 0.77	1.31	
14	(i) $Y = 1.5271x + 3.4503$	1.013 ± 0.078	0.0103	1.5271 ± 0.24	1.00	1.00
	(ii) $Y = 2.9540x + 1.9006$	1.051 ± 0.045	0.0113	2.9540 ± 0.31	1.00	
	(iii) $Y = 4.3869x + 3.8052$	0.271 ± 0.046	0.0187	4.3869 ± 0.88	1.00	

Dates of spraying: (i) 5/1/51, (ii) 21/11/51, (iii) 2/11/51.

Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated lorol.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 6.2-9.5 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 57°F. (before and after spraying); humidity, 60%-70%.

An additional three experiments were carried out in which the developmental period of the eggs was extended to 14 days by lowering the incubation temperature to 57°F. as in the previous experiment with TDNOC. The experimental conditions and details are given in Table V and the relative resistances together with the average relative resistance for each age of egg are given in fig. 5. The general trend in the resistances from the 1-day to the 14-day egg corresponds to that found with 3:5 TDNOC under similar experimental conditions. Somewhat

similar results were obtained by Mukerjea (1953) with pyrethrins against four ages of eggs of *D. oleracea*, i.e., the oldest eggs were the most susceptible.

The temperature at which eggs of *D. oleracea* are incubated and, therefore, the speed of development of the embryo appear to have some effect on the changes of resistance of the egg to both allethrin and TDNOC. A definite resistance/age curve is found with these insecticides at 57°F. (see and compare figs. 2 and 3, 4 and 5).

When using allethrin at 75°F. and at concentrations just sufficient to give 100 per cent. mortality (0.1 per cent.) no effect on the development of the larvae was noticed and many eggs had holes in their shells where the larvae had attempted to hatch. However, as the concentration of allethrin became higher (1.0 per cent. to 1.5 per cent.) more and more of the eggs did not have their shells perforated although they still contained fully developed larvae. Slight movements of these larvae could be seen several hours after control eggs of the same

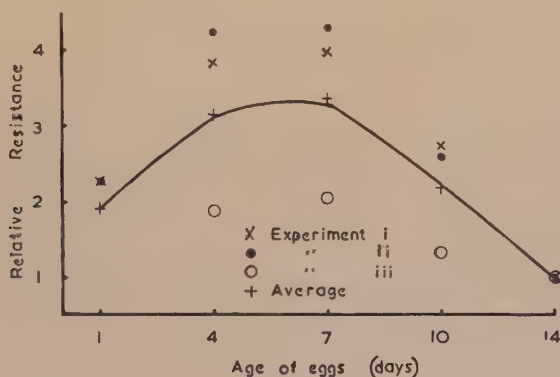


Fig. 5.—Allethrin. The relative resistances of five ages of eggs of *D. oleracea* (three separate experiments) at 57°F. and 60%–70% R.H., and the calculated average value for each age of egg.

age had hatched. A slight slowing up of the developmental process of the embryo was observed by comparing similar ages of both allethrin-sprayed and medium-sprayed eggs throughout their development. The eggs which did not hatch after having been sprayed with a high concentration of allethrin had a greasy mottled brown appearance and contained considerable quantities of a dark brown liquid which surrounded the embryo. As the concentration of allethrin became higher (2.5 per cent. to 5 per cent.), a proportionately greater number of eggs contained either partially developed embryos or yolk cells in which no development could be seen. In such eggs the yolk contents became very dark in colour and quite liquid. This indicated that allethrin was capable of exerting a toxic action before either the respiratory or nervous systems of the embryo were developed. Somewhat similar results were obtained by Potter and Tattersfield (1943) when using pyrethrins against three species of Lepidopterous eggs and by Mukerjea (1953) when using pyrethrins against eggs of *D. oleracea*.

It was noticed in these experiments with allethrin that lower concentrations of the insecticide were required to give a 50 per cent. kill in all ages of eggs at 57°F. than were required to give the same kill at 75°F. (compare Tables IV and V). The LD₅₀ for the youngest and oldest eggs tested at 75°F. was about 5 times greater than that for the youngest and oldest eggs tested at 57°F. A somewhat smaller difference was found for the other ages tested.

TABLE VI.

D. oleracea. The effect of the age of eggs on their susceptibility to HETP. Details of five spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration (i), (ii), (iv), (v) $\times 10^2$ (iii) $\times 10^3$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y=2.0669x + 1.9080$	1.493 ± 0.064	0.311	2.0669 ± 0.41	1.21	1.73
	(ii) $Y=4.2975x - 2.6609$	1.781 ± 0.031	0.604	4.2975 ± 0.55	1.44	
	(iii) $Y=5.7060x - 10.5404$	2.722 ± 0.050	0.527	5.7060 ± 1.92	2.60	
	(iv) $Y=6.4903x - 12.6085$	2.713 ± 0.021	0.516	6.4903 ± 1.14	2.01	
	(v) $Y=6.3336x - 6.2737$	1.780 ± 0.017	0.603	6.3336 ± 0.77	1.73	
2	(i) $Y=3.4777x - 0.6788$	1.632 ± 0.040	0.429	3.4777 ± 0.54	1.66	1.71
	(ii) $Y=6.1867x - 5.9462$	1.769 ± 0.026	0.588	6.1867 ± 1.01	1.41	
	(iii) $Y=3.3500x - 4.0005$	2.687 ± 0.062	0.486	3.3500 ± 0.68	2.39	
	(iv) $Y=4.3617x - 6.4399$	2.624 ± 0.027	0.421	4.3617 ± 0.70	1.68	
	(v) $Y=3.8893x - 1.9618$	1.789 ± 0.028	0.615	3.8893 ± 0.75	1.77	
3	(i) $Y=3.8354x - 0.8533$	1.523 ± 0.025	0.333	3.8354 ± 0.42	1.29	1.35
	(ii) $Y=2.7250x + 0.3761$	1.692 ± 0.033	0.492	2.7250 ± 0.29	1.18	
	(iii) $Y=0.8109x + 2.9750$	2.494 ± 0.21	0.312	0.8109 ± 0.35	1.54	
	(iv) $Y=5.8410x - 9.7910$	2.533 ± 0.032	0.341	5.8410 ± 1.20	1.33	
	(v) $Y=5.4681x - 4.3992$	1.718 ± 0.017	0.522	5.4681 ± 0.55	1.50	
4	(i) $Y=4.6993x - 1.5300$	1.389 ± 0.027	0.245	4.6993 ± 0.61	0.95	1.17
	(ii) $Y=3.1369x - 0.1666$	1.646 ± 0.028	0.443	3.1369 ± 0.28	1.06	
	(iii) $Y=5.8869x - 10.1475$	2.572 ± 0.032	0.373	5.8869 ± 1.04	1.84	
	(iv) $Y=4.9598x - 7.3580$	2.492 ± 0.024	0.311	4.9598 ± 0.77	1.21	
	(v) $Y=5.2868x - 3.2221$	1.554 ± 0.020	0.358	5.2868 ± 0.47	1.03	
5	(i) $Y=4.9242x - 1.9384$	1.411 ± 0.024	0.258	4.9242 ± 0.65	1.00	1.00
	(ii) $Y=4.2543x - 1.8905$	1.621 ± 0.029	0.418	4.2543 ± 0.55	1.00	
	(iii) $Y=3.7722x - 3.6955$	2.308 ± 0.069	0.203	3.7722 ± 0.86	1.00	
	(iv) $Y=5.8755x - 9.1730$	2.410 ± 0.026	0.257	5.8755 ± 1.09	1.00	
	(v) $Y=5.2009x - 3.0191$	1.542 ± 0.039	0.348	5.2009 ± 0.89	1.00	

Dates of spraying: (i) 12/11/50, (ii) 15/12/50, (iii) 1/12/50, (iv) 7/11/50, (v) 23/11/51.

Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated loral.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 8.0-9.6 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after spraying); humidity, 60%-70%.

(d) HETP. Five separate spraying tests were carried out with HETP at 75°F. and 60-70 per cent. R.H. (experiments i, ii, iii, iv, v) the experimental details and the results of which are given in Table VI. The relative resistances and the average relative resistance for each age of egg are given in fig. 6. The general trend of resistance found in the five experiments appears to be a gradual decrease in resistance from the 1-day egg to the 5-day egg, the 1-day egg being approximately 1.7 times more resistant.

In the experiments on comparative resistance of different ages of eggs, HETP did not stop the development of the embryo even at the highest concentration used (1 per cent.) which was more than sufficient to produce a 100 per cent. kill. At this concentration a large percentage of the unhatched eggs had holes in their shells where the larvae had attempted to hatch. However, in a specific test on the effect of the insecticide on embryonic development, a 5 per cent. concentration applied as a spray to the five ages of eggs at 75°F. did prevent the larvae from attempting to hatch although they developed apparently normally until

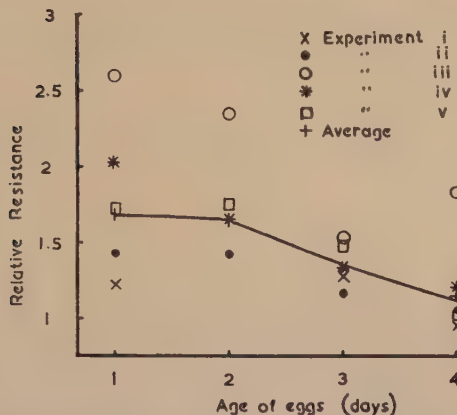


Fig. 6.—HETP. The relative resistances of five ages of eggs of *D. oleracea* (five separate experiments) at 75°F. and 60%–70% R.H., and the calculated average value for each age of egg.

hatching time. Lord and Potter (1951) stated that TEPP-containing materials were toxic to the eggs of *D. oleracea* and *E. künniella* but that high concentrations were required to give a high mortality. They also noticed that these materials were capable of killing the eggs at an early stage in their development. A similar result was found in this work when drops of undiluted HETP containing approximately 15 per cent. TEPP were placed on 1-day-old eggs of *D. oleracea* and the eggs were then incubated at 75°F. Approximately 85 per cent. of the eggs did not develop and although development took place to some extent in the remaining 15 per cent., this development was not normal. The sclerotised and darkened parts of hairs on the abdomen could be distinguished but the embryo

TABLE VII.

E. künniella. The effect of the age of eggs on their susceptibility to the triethanolamine salt of 3:5 dinitro-ortho-cresol. Details of two spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration $\times 10^2$	Median lethal concentration	Slope of probit line (b)	Relative re-sistance	Average relative re-sistance
1	(i) $Y = 4.6456x - 2.6210$	1.639 ± 0.046	0.436	4.6456 ± 0.92	1.49	1.96
	(ii) $Y = 4.8472x - 3.2188$	1.695 ± 0.040	0.496	4.8472 ± 0.95	2.73	
2	(i) $Y = 5.8435x - 5.4164$	1.784 ± 0.027	0.608	5.8435 ± 0.75	2.08	2.67
	(ii) $Y = 7.1887x - 8.0666$	1.818 ± 0.030	0.658	7.1887 ± 1.56	3.62	
3	(i) $Y = 4.0028x - 1.4928$	1.623 ± 0.035	0.420	4.0028 ± 0.65	1.43	1.67
	(ii) $Y = 5.4520x - 3.5669$	1.572 ± 0.028	0.373	5.4520 ± 0.82	2.05	
4	(i) $Y = 3.9813x - 1.3840$	1.603 ± 0.051	0.401	3.9813 ± 0.70	1.37	1.51
	(ii) $Y = 4.2773x - 1.4237$	1.500 ± 0.056	0.316	4.2773 ± 0.97	1.74	
5	(i) $Y = 2.8912x + 0.7566$	1.467 ± 0.055	0.293	2.8912 ± 0.59	1.00	1.00
	(ii) $Y = 3.3509x + 0.7780$	1.260 ± 0.058	0.182	3.3509 ± 0.57	1.00	

Dates of spraying: (i) 15/10/51, (ii) 4/11/51.

Medium: an aqueous medium containing 0.1% sulphonated lorol.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 6.4–7.4 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after spraying); humidity, 60%–70%.

was shrunken and had no definite shape. All the eggs, whether undeveloped or partially developed, were collapsed at the time of inspection although the egg contents were still liquid. The yolk contents of the undeveloped eggs were colourless and the chorion was opaque.

A comparison of the external appearance of eggs killed either with TDNOC, or allethrin or HETP indicates that these three poisons were probably not affecting the embryonic material in the same way.

II. *Ephestia kühniella*.

(a) The triethanolamine salt of 3:5 dinitro-ortho-cresol. Two separate experiments were carried out using 1-day-, 2-day-, 3-day-, 4-day- and 5-day-old eggs. The incubation temperature both before and after spraying was 75°F. and the relative humidity varied between 60 and 70 per cent. The experimental details and the results obtained are given in Table VII. The relative resistances and the average relative resistance for each age of egg are given in fig. 7.

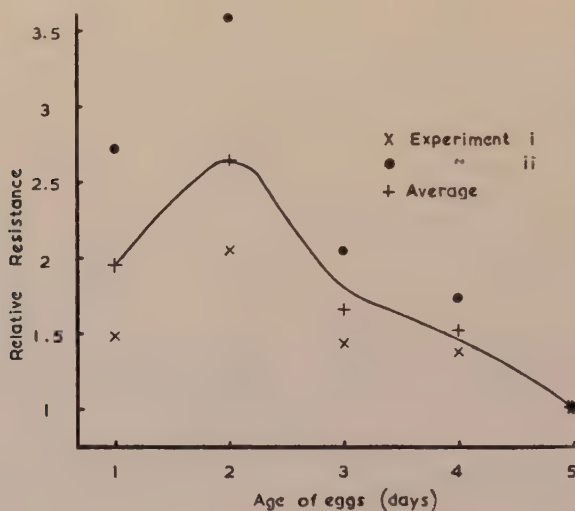


Fig. 7.—TDNOC. The relative resistances of five ages of eggs of *E. kühniella* (two separate experiments) at 75°F. and 60%–70% R.H. and the calculated average value for each age of egg.

Resistance increased by a little more than one-half from the 1-day to the 2-day egg then fell off fairly sharply to the 3-day egg and then more gradually to the 4-day and 5-day eggs. The oldest egg was the most susceptible of the five ages tested and was about twice as susceptible as the youngest egg.

From a comparison of the resistance/age curves for *D. oleracea* and for *E. kühniella* at the same temperature (75°F.) it is obvious that these two species of Lepidoptera vary in their susceptibility to TDNOC although the overall trends of the resistances are somewhat similar.

(b) Allethrin. Two separate experiments were carried out against the same ages of eggs as used in the above tests with TDNOC and under similar conditions of temperature and humidity. The experimental details and results are given in Table VIII and the relative resistances and the average relative resistance found for each age of egg are given in fig. 8. Although there is considerable fluctuation of the relative resistances in the two experiments, the overall trend appears to

TABLE VIII.

E. kühniella. The effect of the age of eggs on their susceptibility to allethrin. Details of two spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration (i) $\times 10^3$ (ii) $\times 10^2$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y=6.9652x - 7.9089$	1.852 ± 0.023	0.071	6.9652 ± 1.43	3.26	2.88
	(ii) $Y=3.7860x + 3.3866$	0.425 ± 0.026	0.027	3.7860 ± 0.46	2.25	
2	(i) $Y=7.3476x - 8.3619$	1.818 ± 0.018	0.066	7.3476 ± 0.93	3.02	3.12
	(ii) $Y=3.8245x + 2.6791$	0.607 ± 0.024	0.040	3.8245 ± 0.44	3.33	
3	(i) $Y=5.2064x - 4.3395$	1.793 ± 0.025	0.062	5.2064 ± 0.86	2.85	2.82
	(ii) $Y=3.7949x + 3.0122$	0.525 ± 0.017	0.034	3.7949 ± 0.32	2.83	
4	(i) $Y=5.9410x - 6.0156$	1.855 ± 0.029	0.070	5.9410 ± 1.50	3.28	2.62
	(ii) $Y=2.9588x + 4.1566$	0.284 ± 0.035	0.019	2.9588 ± 0.29	1.58	
5	(i) $Y=2.2978x + 1.9159$	1.339 ± 0.054	0.022	2.2978 ± 0.34	1.00	1.00
	(ii) $Y=3.8097x + 4.6585$	0.089 ± 0.051	0.012	3.8097 ± 0.52	1.00	

Dates of spraying: (i) 6/10/51, (ii) 3/12/51.
Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated loral.
Total number of eggs used per concentration: 45 (approx.).
Spray deposit: 8.4-9.3 mg./sq. cm.
Replicates: three.
Incubation conditions: temperature, 75°F. (before and after spraying); humidity, 60%-70%.

be very much the same as that found for the TDNOC *i.e.* a small rise in resistance from the 1-day to the 2-day egg followed by a sharp steady decrease in resistance to the 5-day egg which is the least resistant age. The 2-day egg was the most resistant to both allethrin and TDNOC. The difference between the most and the least resistant eggs was about 2.5 times with TDNOC and about 3.0 times with allethrin.

When comparing the results obtained with allethrin against eggs of *E. kühniella* and those obtained with the same insecticide against eggs of *D. oleracea* when the incubation temperature was 75°F., it is apparent that the only difference

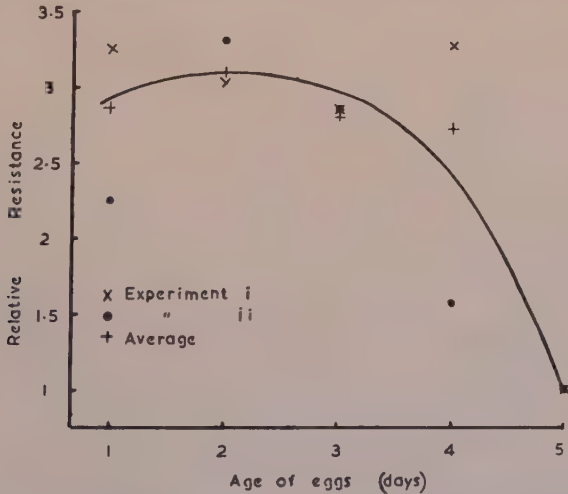


Fig. 8.—Allethrin. The relative resistances of five ages of eggs of *E. kühniella* (two separate experiments) at 75°F. and 60%-70% R.H. and the calculated average value for each age of egg.

in the overall picture is the great decrease in the resistance of the 5-day-old egg of *E. kühniella* to the allethrin.

(c) HETP. Three separate tests were carried out with this insecticide under experimental conditions similar to those used with the two other insecticides. The results obtained together with the experimental details are given in Table IX. The relative resistances and the average relative resistance found for each age of egg are given in fig. 9. Susceptibility to HETP at 75°F. appears to increase gradually from the youngest to the oldest egg and this trend is almost identical with that found for HETP against eggs of *D. oleracea* at the same temperature. The difference between the most and the least resistant eggs in the former case (*E. kühniella*) was about 2.5 times and was about 1.5 times in the latter case (*D. oleracea*).

III. *Dysdercus fasciatus*.

(a) The triethanolamine salt of 3:5 dinitro-ortho-cresol. Four separate experiments (experiments i, ii, iii, iv) were carried out using 1-day-, 5-day-, and 8-day-old eggs at an incubation temperature of 75°F. The resistance to this insecticide increased with the age of the egg. This increase was only slight between the 1-day and the 5-day eggs but was much larger between the 5-day and the 8-day egg (see fig. 10). To determine the age at which these eggs began to increase more rapidly in resistance, an experiment was carried out (experiment v) with six different ages of eggs *i.e.* 3-, 4-, 5-, 6-, 7- and 8-day-old eggs. From the results obtained it would appear that resistance begins to increase with the 5-day egg and rises steadily to the 8-day egg. The experimental details of all five experiments together with the results obtained are given in Table X and the relative

TABLE IX.

E. kühniella. The effect of the age of eggs on their susceptibility to HETP. Details of three spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration $\times 10^2$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y = 1.3795x + 2.7602$	1.623 ± 0.066	0.420	1.3795 ± 0.31	3.21	2.32
	(ii) $Y = 4.2828x - 3.0129$	1.871 ± 0.017	0.743	4.2828 ± 0.45	1.62	
	(iii) $Y = 3.2331x - 0.9434$	1.839 ± 0.016	0.690	3.2331 ± 0.52	3.29	
2	(i) $Y = 5.0321x - 2.9810$	1.586 ± 0.029	0.386	5.0321 ± 0.75	2.95	2.47
	(ii) $Y = 6.5579x - 7.4659$	1.901 ± 0.014	0.796	6.5579 ± 0.80	1.73	
	(iii) $Y = 5.2673x - 5.0116$	1.899 ± 0.013	0.793	5.2673 ± 0.81	3.78	
3	(i) $Y = 2.9873x + 0.3535$	1.555 ± 0.043	0.359	2.9873 ± 0.43	2.74	2.20
	(ii) $Y = 8.3426x - 10.3436$	1.839 ± 0.010	0.690	8.3426 ± 0.68	1.50	
	(iii) $Y = 7.5652x - 9.0269$	1.853 ± 0.010	0.713	7.5652 ± 0.82	3.40	
4	(i) $Y = 2.8820x + 0.9190$	1.417 ± 0.043	0.261	2.8820 ± 0.40	1.99	1.53
	(ii) $Y = 7.8535x - 9.2445$	1.814 ± 0.011	0.652	7.8535 ± 0.71	1.42	
	(iii) $Y = 3.4308x - 0.1141$	1.490 ± 0.018	0.309	3.4308 ± 0.15	1.47	
5	(i) $Y = 2.0300x + 2.7266$	1.118 ± 0.080	0.131	2.0300 ± 0.36	1.00	1.00
	(ii) $Y = 7.6432x - 7.6993$	1.662 ± 0.014	0.459	7.6432 ± 0.82	1.00	
	(iii) $Y = 3.6248x + 0.2125$	1.323 ± 0.062	0.210	3.6248 ± 0.73	1.00	

Dates of spraying: (i) 6/11/51, (ii) 23/11/51, (iii) 25/11/51.

Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated lorol.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 6.4–10.9 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after spraying); humidity, 60%–70%.

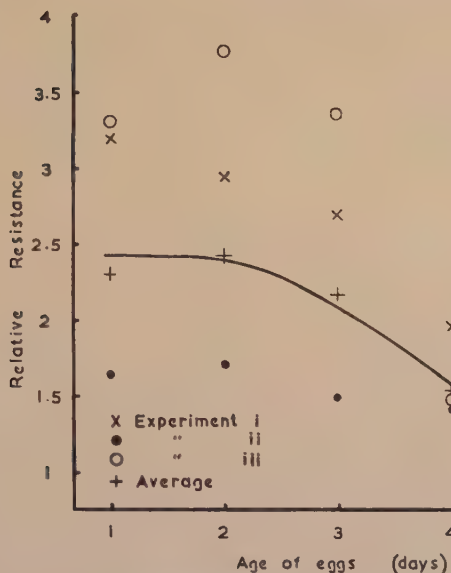


Fig. 9.—HETP. The relative resistances of five ages of eggs of *E. künniella* (three separate experiments) at 75°F. and 60-70 per cent. R.H., and the calculated average value for each age of egg.

TABLE X.

D. fasciatus. The effect of the age of eggs on their susceptibility to the triethanolamine salt of 3:5 dinitro-ortho-cresol. Details of five spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration (i), (iii), (iv), (v) $\times 10^2$ (ii) $\times 10^3$	Median lethal concentration	Slope of probit line (b)	Relative resistance	Average relative resistance
1	(i) $Y = 2.4272x + 1.9385$	1.259 ± 0.147	0.182	2.4272 ± 0.81	0.44	0.33
	(ii) $Y = 1.9045x + 0.7723$	2.226 ± 0.053	0.168	1.9045 ± 0.26	0.41	
	(iii) $Y = 3.4749x + 1.1931$	1.098 ± 0.053	0.125	3.4749 ± 0.65	0.24	
	(iv) $Y = 2.8035x + 1.6792$	1.186 ± 0.059	0.154	2.8035 ± 0.43	0.28	
3	(v) $Y = 3.3362x + 0.8762$	1.234 ± 0.043	0.171	3.3362 ± 0.42	0.36	0.36
4	(v) $Y = 3.8988x + 0.0146$	1.279 ± 0.043	0.190	3.8988 ± 0.56	0.40	0.40
5	(i) $Y = 3.9066x - 0.8310$	1.491 ± 0.033	0.310	3.9066 ± 0.54	0.75	0.49
	(ii) $Y = 1.6309x + 0.9996$	2.381 ± 0.070	0.240	1.6181 ± 0.32	0.59	
	(iii) $Y = 3.6370x + 0.9493$	1.113 ± 0.052	0.130	3.6370 ± 0.61	0.25	
	(iv) $Y = 2.2278x + 1.8101$	1.430 ± 0.048	0.269	2.2278 ± 0.28	0.49	
	(v) $Y = 3.1917x + 0.9072$	1.282 ± 0.037	0.191	3.1917 ± 0.38	0.41	
6	(v) $Y = 3.8971x - 0.5654$	1.428 ± 0.058	0.268	3.8971 ± 0.84	0.57	0.57
7	(v) $Y = 3.8322x - 1.1282$	1.601 ± 0.032	0.399	3.8322 ± 0.50	0.85	0.85
8	(i) $Y = 5.1484x - 3.3147$	1.614 ± 0.023	0.411	5.1484 ± 0.55	1.00	1.00
	(ii) $Y = 6.1016x - 10.9155$	2.610 ± 0.029	0.407	6.1016 ± 1.44	1.00	
	(iii) $Y = 1.9315x + 1.6995$	1.710 ± 0.041	0.513	1.9315 ± 0.19	1.00	
	(iv) $Y = 3.4593x - 1.0077$	1.737 ± 0.030	0.546	3.4593 ± 0.52	1.00	
	(v) $Y = 2.0450x + 1.5718$	1.673 ± 0.034	0.471	2.0450 ± 0.23	1.00	

Dates of spraying: (i) 12/9/51, (ii) 10/10/51, (iii) 15/10/51, (iv) 4/11/51, (v) 6/12/51.

Medium: an aqueous medium containing 0.1% sulphonated lorel.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 6.0-8.6 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after spraying); humidity, saturated atmosphere.

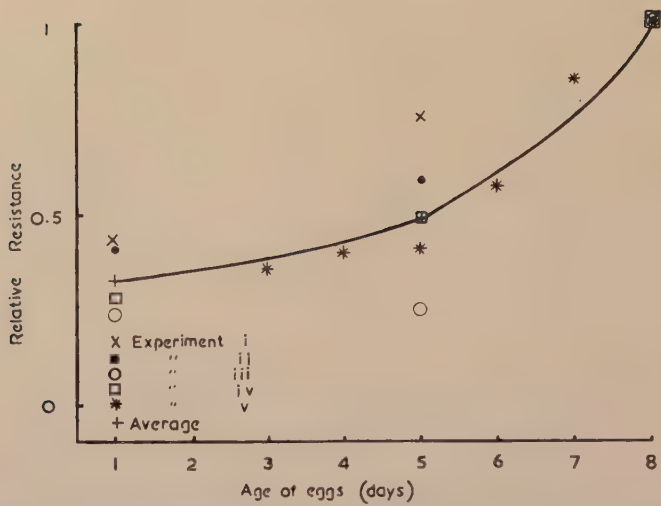


Fig. 10.—TDNOC. The relative resistances for various ages of eggs of *D. fasciatus* (five separate experiments) at 75°F. in a saturated atmosphere, and the calculated average value for each age of egg.

resistances and the average relative resistance found for each age of egg tested are shown in fig. 10. It is of interest to note that the trend of the relative resistances for the different ages of eggs of *D. fasciatus* when using TDNOC as the insecticide

TABLE XI.

D. fasciatus. The effect of the age of eggs on their susceptibility to allethrin. Details of three spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration $\times 10^2$	Median lethal concentration	Slope of probit line (b)	Relative re-sistance	Average relative re-sistance
1	(i) $Y=1.1487x + 2.9587$ (ii) $Y=1.2827x + 1.6448$	1.774 ± 0.13 2.625 ± 0.11	0.59 4.22	1.1487 ± 0.39 1.2827 ± 0.31	0.20 0.40	0.35
4	(i) $Y=2.3684x + 0.5356$ (iii) $Y=2.3886x + 0.3373$	1.882 ± 0.085 1.950 ± 0.079	0.76 0.89	2.3684 ± 0.70 2.3886 ± 0.43	0.26 0.13	0.12
5	(i) $Y=0.9484x + 3.3491$ (ii) $Y=1.3766x + 1.4185$ (iii) $Y=1.8861x + 1.1133$	1.737 ± 0.21 2.594 ± 0.08 2.058 ± 0.085	0.55 3.93 1.14	0.9484 ± 0.50 1.3766 ± 0.27 1.8861 ± 0.39	0.18 0.37 0.17	0.27
6	(i) $Y=1.6390x + 1.6765$ (iii) $Y=1.1891x + 2.2125$	2.024 ± 0.088 2.345 ± 0.059	1.06 2.21	1.6390 ± 0.60 1.1891 ± 0.18	0.36 0.32	0.24
7	(iii) $Y=1.6670x + 0.8682$	2.473 ± 0.057	2.97	1.6670 ± 0.28	0.43	0.43
8	(i) $Y=1.5946x + 1.0693$ (ii) $Y=2.2852x - 1.9268$ (iii) $Y=1.5812x + 0.5198$	2.472 ± 0.14 3.026 ± 0.20 2.835 ± 0.12	2.97 10.62 6.84	1.5946 ± 0.57 2.2852 ± 0.94 1.5812 ± 0.43	1.00 1.00 1.00	1.00

Dates of spraying: (i) 23/10/51, (ii) 2/11/51, (iii) 11/12/51.
Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated lorol.
Total number of eggs used per concentration: 45 (approx.).
Spray deposit: 6.4-8.6 mg./sq. cm.
Replicates: three.
Incubation conditions: temperature, 75°F. (before and after spraying);
humidity, saturated atmosphere.

is the reverse of that found with the same insecticide for eggs of *E. kühniella* at 75°F. and for those of *D. oleracea* at 57°F.

(b) Allethrin. Three experiments were carried out at 75°F. using various ages of eggs. The experimental details and the results obtained are given in Table XI and the relative resistances together with the average relative resistance for each age of egg are given in fig. 11. Resistance appears to drop very slightly

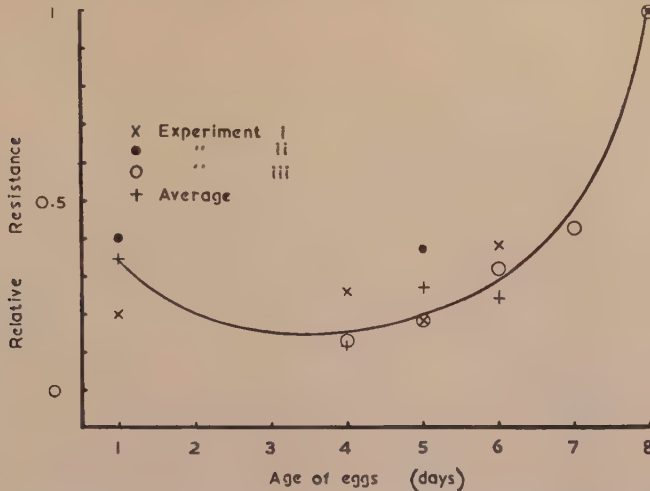


Fig. 11.—Allethrin. The relative resistances of various ages of eggs of *D. fasciatus* (three separate experiments) at 75°F. in a saturated atmosphere, and the calculated average value for each age of egg.

from the 1-day- to the 4-day-old egg and then increases quite rapidly to the 8-day-old egg. Once again the general shape of the resistance/age curve obtained for the eggs of *D. fasciatus* is the reverse of that found for eggs of *E. kühnliella* at 75°F. and for eggs of *Diataraxia oleracea* at 57°F. when using the same insecticide, allethrin. It seems possible that these differences in the resistance/age

TABLE XII.

D. fasciatus. The effect of the age of eggs on their susceptibility to HETP. Details of two spraying tests.

Age of eggs (days)	Regression equation	Log. median lethal concentration $\times 10^2$	Median lethal concentration	Slope of probit line (<i>b</i>)	Relative resistance	Average relative resistance
1	(i) $Y = 2.2167x - 1.3715$ (ii) $Y = 3.0077x - 3.6177$	2.869 ± 0.12 2.864 ± 0.082	7.40 7.31	2.2167 ± 0.75 3.0077 ± 1.22	7.96 14.33	10.22
5	(i) $Y = 2.3117x - 1.4430$ (ii) $Y = 1.8310x + 0.0848$	2.788 ± 0.25 2.689 ± 0.053	6.14 4.89	2.3117 ± 2.45 1.8310 ± 0.27	6.61 9.59	7.66
8	(i) $Y = 2.8003x - 0.5149$ (ii) $Y = 1.7873x + 1.9363$	1.968 ± 0.061 1.709 ± 0.063	0.93 0.51	2.8003 ± 1.26 1.7873 ± 0.32	1.00 1.00	1.00

Dates of spraying: (i) 6/11/51, (ii) 25/11/51.

Medium: an aqueous medium containing 10% acetone and 0.1% sulphonated lorol.

Total number of eggs used per concentration: 45 (approx.).

Spray deposit: 9.0-11.0 mg./sq. cm.

Replicates: three.

Incubation conditions: temperature, 75°F. (before and after spraying); humidity, saturated atmosphere.

relationship between the two insect orders could be caused, at least in part, by the different methods of hatching occurring in the two orders. The egg of *D. fasciatus* splits open, freeing the nymph, but the larvae of the Lepidoptera hatch by eating their way out of the shell and in so doing are more likely to become exposed to or ingest any toxic substance that might be either in or on the egg-shell. This fact may have some bearing on the high resistance found in the oldest egg of *D. fasciatus* and the low resistance found in the oldest egg of *D. oleracea*.

(c) HETP. Two experiments using 1-day-, 5-day- and 8-day-old eggs were carried out at 75°F. The experimental details and results are given in Table XII and the relative resistances together with the average relative resistance found for each age of egg are given in fig. 12. In contrast to the order of the relative resistances found for the different ages of eggs when using allethrin and TDNOC, resistance to HETP decreases with age. Indeed the 1-day-old egg is about 10 times as resistant as the 8-day-old egg. It is well known that HETP hydrolyses quickly to relatively non-toxic substances in the presence of water, and it seems possible that because the eggs were incubated in a saturated atmosphere, some of the HETP had become hydrolysed and was ineffective as a poison.

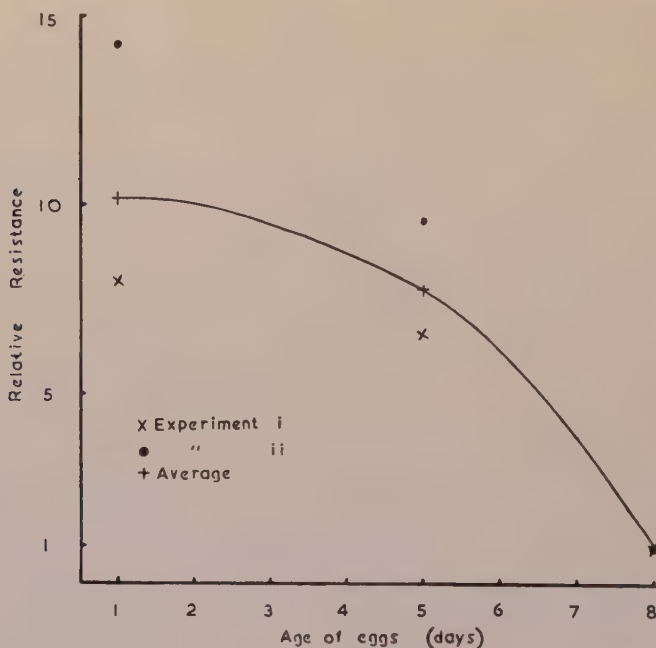


Fig. 12.—HETP. The relative resistances of three ages of eggs of *D. fasciatus* (two separate experiments) at 75°F. in a saturated atmosphere, and the calculated average value for each age of egg.

A similar resistance/age relationship to HETP although not so marked, was found with the eggs of the two species of Lepidoptera tested.

Structure and Permeability of the Chorion and Embryonic Membranes of Eggs of *D. oleracea*.

A fairly detailed description of the external appearance of this egg has been given by Lloyd (1920). The egg is hemispherical or dome-shaped and has a

diameter of about 0.75 mm. The shell is strongly ribbed except for the floor which is flat and smooth. The most prominent of the ribs extend from the outer edge of the floor and converge into a fan-like pattern about the apex. There are less conspicuous ribs running horizontally around the circumference of the shell and joining with the vertical ribs. The apex or dome is slightly depressed and the funnel-shaped cavity at the extreme upper pole contains the micropylar structure.

When newly laid, the egg has a green colour caused by the pigmentation of the yolk; the shell itself is almost transparent. When the egg is incubated at 75°F., the larva hatches in just over 5 days. No evidence of the development of the embryo can be seen from an external examination of the shell until the egg is about 3 days old, although the yolk gradually becomes a more yellowish-green colour. Indeed, no development of the embryo was apparent, even when the yolk was dissected, until the egg was about 60 hours old. At this time, some of the yolk cells had become differentiated but no definite larval form could be identified. When the egg is about 3 days old a dark V-shaped spot which is one of the larval eyes appears near the apex and can be plainly seen through the shell. After an additional period of about 12 hours, another similar spot can be seen a short distance below the other one. When an egg of this age (84 hours old) was dissected the embryo was found to be partially developed, *i.e.*, the outline of the embryo was apparent, but segmentation of the appendages had not taken place, and the embryo had a worm-like appearance. About 4 days after the egg has been laid, black dot-like sclerites and hairs on the abdomen can be seen through the chorion. At this time also, the head has become a dark brown and the whole egg is of a greyish colour although part of the muddy-green-coloured yolk still remains near the apex. In addition the larva is capable of movement and as hatching becomes imminent (120 hours), the yolk entirely disappears and the complete outline of the larva can be seen through the chorion.

Sectioning methods.

A picture of the general shell structure was obtained from gross sections of the shell, staining reactions of different layers of the shell by both water- and fat-soluble stains, the corrosive action of strong acids and alkalis and results of qualitative biochemical tests on the egg-shell and the penetration studies of the egg as a whole. The intention of this present work was to study only the gross structure of the chorion and other protective envelopes in an attempt to determine how they might affect the penetration of insecticides to the embryo. It should be pointed out, therefore, that considerably more detailed micro-chemical tests will have to be carried out before the complete structure of the shell of this egg can be determined.

Sections of the shell were obtained by the freezing technique described by Beament (1946a). Whole eggs were orientated in a small drop of either water or glycerine jelly, frozen with ethyl chloride and cut in halves with a razor blade. The yolk contents were washed out with water and the half-shells were then dried on filter paper, stained, mounted in a cavity slide in a medium in which the particular stain used was not miscible, and viewed under oil immersion. When studying the staining reaction of the inner layers of the shell, the stain was dropped into the shell cavity with a very fine glass pipette. After the half-shells had been stained, they were again cut in halves, and mounted on slides so that the freshly cut edge was in contact with the cover slip. In this way the exact amount of penetration of the stain through the shell could be ascertained. Euparal was used as the mounting medium with water-soluble stains and glycerine was used for fat-soluble stains.

Although the formation of the chorion has not been followed layer by layer as it was laid down by the follicle cells, unlaidd and therefore unfertilised eggs were examined to determine the layers present at this time and to compare these layers with those found in the developing eggs. In this way a distinction between that part of the shell formed by the follicle cells and that part formed by the developing embryo could be made. The former part of the shell will be termed the chorion and the latter part the embryonic or sub-chorial membranes as Beament (1946a) has suggested.

It has been generally agreed by insect embryologists that the chorion of most insect eggs is primarily a double-layered structure composed of an exochorion and an endochorion. Beament (1946a) has subsequently sub-divided these layers in the egg of *Rhodnius prolixus* into at least seven secondary layers. In this present investigation of the structure of the shell of the egg of *Diataraxia oleracea* the methods used are much the same as those used by Beament (1946a, 1948, 1949) in his study of the structure and permeability of the egg of *R. prolixus*.

Shell structure of an ovarian egg.

Each of the two ovaries of *D. oleracea* consist of four polytrophic ovarioles. When the chorion is completely formed by the follicle cells of the ovarioles the eggs are released into the pedicles and then into the common oviduct where they are stored until laid. They are fertilised as they pass the opening of the *ductus receptaculi* immediately before they are laid (cf. Norris, 1932, and Musgrave, 1937). Thus all eggs are at approximately the same stage of development when they are laid.

Because of the ribbed structure of the chorion, its overall thickness varies considerably. It was found to be approximately 3 microns thick between the ribs and about 7 microns thick through the ribs. When an ovarian egg is dissected and the yolk washed out, a thin gelatinous-like membrane remains attached to the inner surface of the shell. This membrane can be removed in small pieces by careful scraping with a needle after the shell has been soaked in water, but if the shell is first soaked in a wax solvent such as acetone or xylol for a few moments, the membrane floats away from the shell in a continuous sheet. This would seem to indicate that some sort of a weak wax-like bond was holding this membrane to the inner surface of the shell. The membrane is about 0.7 microns thick and stains fairly heavily in water-soluble stains and more lightly in fat-soluble stains. The stains used were:—basic fuchsin in water, fast green in water, methylene blue in water, basic fuchsin in 70 per cent. alcohol, and Sudan III and Sudan black both in 70 per cent. alcohol.

This is probably a proteinaceous membrane because it gave a weak ninhydrine test as well as a weak positive polyphenol test with 5 per cent. ammoniacal silver nitrate (Lison, 1936). Colour reactions such as these, however, were very difficult to judge because of the thinness of the membrane. The protein is in all probability tanned because the membrane is insoluble in cold concentrated hydrochloric and sulphuric acids and in cold potash, but is slowly dissolved in hot nitric acid and in chlorinated nitric acid. It seems likely that no lipoids were present since no oil droplets appeared to be released as the membrane dissolved.

This layer probably corresponds to the vitelline membrane found in eggs of many other species of insects. Indeed, its chemical and physical properties bear a striking resemblance to those of the vitelline membrane in the egg of *R. prolixus* (Beament, 1946a). However, as described later, certain changes occur in this membrane in the laid egg which indicate that at a particular stage of egg development it is probably a composite structure.

When the vitelline membrane has been removed, either by scraping or with a wax solvent, the exposed inner surface of the shell is very hydrophobic, and

remains so even after it has been soaked in cold wax solvents for several hours. Neither water- nor fat-soluble stains give any appreciable staining reaction when dropped into the shell cavity. If the shell is placed in cold nitric acid it gradually but completely dissolves except for a thin (approximately 0.5 microns) innermost layer. This layer is also insoluble in concentrated sulphuric and hydrochloric acids and cold potash, but can be broken down in hot chlorinated nitric acid. Its properties seem to be very similar to those found for the vitelline membrane but it appears to be more completely tanned and impermeable. This layer will be called the resistant endochorion through this work.

The rest of the chorion can be divided into two layers by its solubility in cold potash and in nitric acid. When a whole egg is placed in nitric acid an immediate reaction takes place and a very thin outer layer of the shell swells up, giving the egg a blistered appearance. This layer dissolves slowly, giving off oily droplets which will stain pink with Sudan III in 70 per cent. alcohol, indicating that lipoids are present. After an egg has been soaked in cold potash for several hours, an outer very thin layer becomes separated from the rest of the shell and can be ripped off in small pieces with a needle. It still retains the sculptured appearance of the shell and stains faintly in Sudan black, a fat-soluble stain, as well as with water-soluble stains. This staining is especially noticeable in the ribs. It seems likely that this layer may be composed of lipoproteins and its position and properties suggest that it is the exochorion (see Beament, 1946a).

The remaining central part of the shell is about 2 microns thick but this thickness becomes greater in the region of the ribs. It retains the sculptured design of the shell although much less sharply outlined. It stains quite readily in water-soluble stains but only very slightly, if at all, in lipid-soluble ones. It is fairly soluble in nitric acid but becomes brittle in cold potash and tough and elastic in sulphuric acid. It would appear to be composed of some type of modified protein.

Although protein tests were not carried out on the individual layers of the chorion (with the exception of the vitelline membrane), tests were done on the shell as a whole. A fairly strong proteinaceous reaction was observed (ninhydrine, xanthoproteic and arginine). In addition, a test for chitin (Campbell, 1929) was undertaken with negative results. Whole eggs were treated with an enzyme solution containing chitinase but by testing with a colorimetric method no chitin was found.

Thus the chorion of the ovarian egg of *D. oleracea* appears to be composed of at least five layers (see and compare fig. 13).

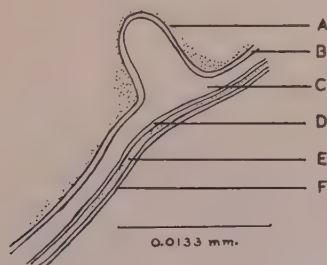


Fig. 13.—Diagrammatic cross-section of the chorion of the egg of *D. oleracea* indicating the approximate relative thickness of the layers. (A) cement layer, (B) exochorion, (C) soft protein layer, (D) resistant endochorion, (E) wax layer, (F) vitelline-fertilisation membrane.

- (1) The exochorion, probably composed of lipoproteins.
- (2) A soft protein layer, approximately 2 microns thick.

- (3) The resistant endochorion, a fairly thin (0.5 microns) proteinaceous layer, the internal surface of which is very hydrophobic.
- (4) A "waterproofing" layer composed of wax-like material.
- (5) A vitelline membrane.

The egg is waterproofed before it is laid. Indeed, waterproofing has taken place in eggs removed just below the *corpus luteum* and these eggs can withstand desiccation for a few days at a relative humidity below 50 per cent. Eggs from the ovarioles collapse rapidly when exposed to such humidity conditions. The waterproofing mechanism appears to be the wax layer complex between the resistant endochorion and the vitelline membrane.

If water-soluble stains are dropped into half-shells only the vitelline membrane becomes stained. Similarly, if whole eggs are immersed in an aqueous stain solution for several hours, the chorion becomes stained but the vitelline membrane remains unstained. However, if the wax layer is first disrupted by soaking the eggs in acetone for a few minutes and the eggs are then immersed in the stain solution, the shell becomes permeable and stain penetrates to the vitelline membrane and the yolk. A stain such as basic fuchsin in 70 per cent. alcohol can penetrate the shell and stain the yolk without the egg having been treated in a wax solvent. Thus the wax solvent properties of the 70 per cent. ethyl alcohol appear to be sufficiently efficient to disrupt the protective wax layer of the egg.

Shell structure of the laid egg.

Eggs from each age group used in the spraying experiments were studied. The most noticeable difference between an ovarian egg and a laid egg is the very hydrophobic external surface of the latter. This property is the result of a secretion which is expressed on to the chorion from the colleterial glands immediately after the egg has become fertilised and just as it is being laid. This material is generally referred to as cement and serves to attach the eggs to the substrate on which they are laid (*cf.* Norris, 1932). The cement is a very viscous liquid which becomes hardened when exposed to air. When soaked in water it becomes slowly softened and viscous again but it is not soluble in water. It is soluble in wax- and fat-solvents such as acetone and the shell is immediately wetted when immersed in such a solution. When fresh cement material is dissected from the cement glands of the female, it dries quickly in air and stains very heavily in water-soluble and alcoholic stains but not in fat-soluble stains. It is soluble in cold nitric acid and potash but no oil droplets appeared to be released. It seems probable that the cement is composed of some type of proteinaceous material. The cement covers the whole surface of the egg but collects in large quantities about the base of the ribs. Although the cement appears to be permeable to both hydrophiles and lipophiles, its initial hydrophobic properties would probably be a considerable hindrance to the penetration of hydrophilic liquids.

When eggs of *D. oleracea* are placed in a hydrophilic liquid they float unless completely submerged by mechanical action. Submerged eggs still retain their hydrophobic properties for a considerable time and bubbles of air are trapped between the ribs of the egg-shell. There is therefore only a limited area of the shell where there is a liquid-solid interface. This area can be increased by using a wetter, such as sulphonated lorol, in the spray solutions. In the course of preparing different aged eggs for the spraying experiments, it was noticed that the 1-day-old eggs could be more easily separated from the batches in which they were laid by soaking in water than the older eggs. The cement appeared to become slightly harder and more brittle with age. However, this increase in hardness of the cement is only very slight and probably has little influence on the permeability of the shell. Beament (1948) when describing the cement layer

on eggs of *Rhodnius prolixus* observed that there was an uneven distribution of cement on eggs of the same age chosen at random and he concluded that this might be one factor accounting for deviations between replicates in an ovicidal experiment. No such great differences in the distribution and amount of cement on similar aged eggs of *D. oleracea* were noticed. The randomisation of the eggs of any one age and the three replicates used in each spraying experiment would presumably eliminate any noticeable differences in susceptibility due to this factor.

With the exception of the cement layer which has been described above, the microscopical and chemical properties of the chorion of the laid egg appear to be almost identical to those of the ovarian egg (fig. 13) but some changes do occur in the vitelline membrane. It has been mentioned previously that this membrane in ovarian eggs becomes stained in water- and fat-soluble stains. This staining is only slight over the whole area of the membrane except around the micropylar area where much heavier staining takes place. In the 1-day-old egg this darkly staining area becomes more clearly defined as discrete dark spots which have darkly staining tube-like structures connecting them and also radiating outwards towards the yolk as well as inwards towards the apex of the egg (fig. 14). It seems possible that these structures may be connected in some way with the fertilisation of the egg since they appear only after fertilisation has taken place. No fertilisation membrane similar to that described by Beament (1949) in eggs of *R. prolixus* was found in the eggs of *D. oleracea*. The physical and chemical properties of the vitelline membrane did not change in any perceptible manner after fertilisation except for those changes mentioned

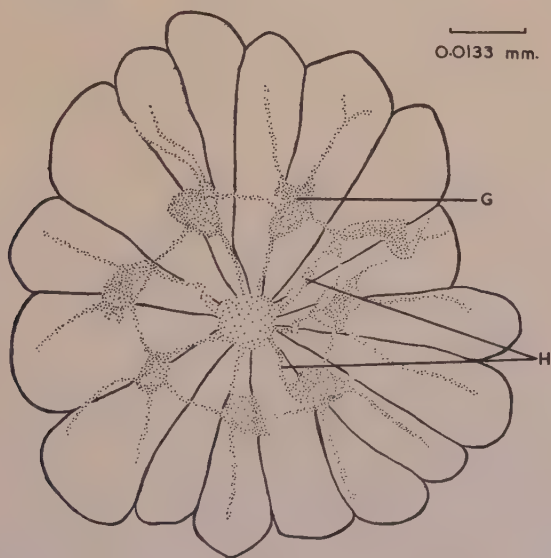


Fig. 14.—Internal surface of the micropylar area (apex) of the egg of *D. oleracea* showing the darkly staining structures (G) on the vitelline-fertilisation membrane and the canals (H), which may lead to the micropyle.

above. However, because such changes do occur, it was decided to rename the vitelline membrane the "vitelline-fertilisation membrane" in the laid eggs. From such a cursory examination of this membrane as was carried out in this work, it was difficult to decide whether these darkly staining structures were merely modifications of the already existing vitelline membrane or were actually formed by additional material from the yolk cells. Because of the lack of

definite information on this point, the vitelline-fertilisation membrane will be considered as part of the chorion. Korschelt (1884) when describing the micropylar structure in the egg of *Sphinx ligustri* L. also found structures somewhat similar to those described above.

Formation of membranes by the oöcyte.

A study of the sub-chorial membranes found in the five different ages of eggs used in the spraying tests indicated that certain changes occurred in this region. No definite membrane surrounding the yolk and separate from the chorion could be found in the 1-day egg by dissection methods. The yolk cells appeared to be in close contact with the vitelline-fertilisation membrane. The formation of a membrane which is probably the serosa was first noticed in the 2-day egg. The formation and general structure of the serosal membrane in a typical Lepidopteran *Diacrisia virginica* (F.) has been described by Johannsen and Butt (1941) and is, according to them, a derivative of the extraembryonic primary epithelium (blastoderm). In the 2-day-old egg of *Diataraxia oleracea* this membrane was composed of elongated very thin cells scattered about the surface of the yolk. It became tougher, thicker and less easily torn as the egg developed and appeared to reach its maximum thickness when the egg was about 4 days old. The serosal membrane stained heavily in both water- and fat-soluble stains and gave a positive ninhydrine test for protein and was probably composed, therefore, of lipoproteins.

Soon after the formation of the serosa, a viscous liquid-like substance was formed which separated the serosal membrane from the chorion. This material was quite elastic and appeared to be attached to the serosa as well as to the vitelline-fertilisation membrane, and became more copious as the serosa developed. The embryo and the surrounding yolk material enclosed within the serosa could be removed intact from the chorion after the egg was between 3 and 4 days old. If the embryo enclosed within the serosa was then incubated on a moist filter paper in a saturated atmosphere at 75°F., development proceeded apparently normally and the embryo hatched, i.e., consumed all the surrounding yolk as well as the serosa. At any lower humidity desiccation of the embryo occurred.

The changes which appear to take place in the protective envelopes of the developing egg of *D. oleracea* are, for the most part, those connected with the embryonic or sub-chorial membranes. The structural and chemical properties of these membranes would seem to indicate that they do not offer a great deal of further protection to the embryo either from hydrophilic or lipophilic liquids, although the presence of these membranes might increase the time required for an insecticide to penetrate to its site of action if that site of action were the embryo itself.

Beament (1946a) has suggested that a secondary wax layer is produced at a definite time during the development of eggs of *R. prolixus* and that this additional material is partly responsible for the increased resistance of the eggs at that particular time. By finding the transition temperature of the different ages of eggs (the temperature at which desiccation of the egg increases sharply) the age of the egg at which this secondary wax layer was laid down could be found because the transition temperature would presumably increase at this time. Although no indication of the presence of such a layer was noticed in the chemical studies on the shell of eggs of *D. oleracea*, it was considered advisable, as a further test, to determine the transition temperature of these eggs at different times during their development.

Single eggs of known age were immersed in a saturated solution of sodium chloride and the solution was gradually heated in a liquid paraffin bath. This process was watched under a binocular microscope and the temperatures at which the eggs collapsed were recorded. After each observation the saline solution was discarded and a fresh solution of the same concentration used for

the next observation. This method was similar to that described by Beament (1951) for determining the transition point of the waterproofing wax layer in the egg of the fruit tree red spider mite, *Metatetranychus ulmi*. The rate of collapse of the eggs of *D. oleracea* was quite rapid at the transition temperature indicating that the orientation of the wax layer molecules had been disrupted and the egg was no longer waterproofed (cf. Beament, 1946b, 1949; Wigglesworth, 1945; and Davies, 1948). The results obtained are given in Table XIII.

TABLE XIII.

D. oleracea. Transition temperature of eggs of different ages, incubated at 75°F.

Age of egg	Number of eggs	Average transition temperature °F.
Ovarian egg	16	61.8
1 day	42	61.7
2 "	46	62.8
3 "	26	63.8
4 "	26	63.0
5 "	26	63.1

It can be seen that the transition temperature remains relatively constant throughout the development of the egg and that this temperature is fairly high for insect waxes (Beament, 1945). Beament also states that the higher the melting point the more waterproof the wax. It has been shown by Way and



Fig.15.—External surface of the micropylar area (apex) of the egg of *D. oleracea* showing the external openings of the micropyle (I).

others (1951) that eggs of *D. oleracea* are not affected by humidity changes and that even at 0 per cent. R.H. a hatch of 93 per cent. was obtained. It would appear, therefore, that the eggs of *D. oleracea* are very effectively waterproofed. The slightly higher transition temperatures for the older eggs may be due to the

extra structural rigidity of the embryo which would retard the collapse of the shell.

Penetration experiments with the eggs of Diataraxia oleracea.

From a study of the egg-shell of *D. oleracea* it is recognised that specialised areas of the shell such as the micropylar canals which penetrate through certain layers of the chorion may facilitate the entry of poisons to the embryonic material. Indeed, Beament (1948) has shown that a range of materials with widely differing properties enter the egg of *Rhodnius prolixus* only through the micropyles and not through the general shell surface. If this is true of the egg of *D. oleracea*, then it is obvious that the position of the egg at the time of spraying would have to be such that the liquid was in contact with the micropyle. It therefore seemed of importance to determine if insecticides such as those previously used in the spraying experiments could penetrate to the embryo only through the micropylar area.

The micropyle, as previously mentioned, is situated in the funnel-shaped cavity of the extreme upper pole of the egg. It is a complex structure composed of from 6–8 micropylar canals (figs. 14 and 15) which radiate from this depression outwards and downwards through the chorion possibly to the vitelline-fertilisation membrane. Leuckart (1855) has given a detailed description of the micropylar structure in the eggs of several Sphingid species which appear to be very similar to that found in eggs of *D. oleracea*.

In this present work attempts were made to seal off the micropylar area of the egg with an impermeable material and then to carry out dipping tests with these eggs to determine the importance of this area of the egg in the penetration of liquids through the chorion. Materials such as paraffin wax (low melting point), celluloid solution and Canada balsam were tried but because of the very hydrophobic and strongly ribbed surface of the shell none of these materials would form a water-tight seal with the shell. A drop technique was finally used in which a small drop (0.0007 cc.) of the insecticide in distilled water was placed, either on the apex (micropyle) of the egg or on the floor of the egg, with a very fine needle on a hypodermic syringe. These drops were small enough and the surface of the shell was sufficiently hydrophobic so that they did not appear to spread over the surface of the shell, although it seems possible that because of the water-absorbing property of the cement layer, some of the insecticide solution may have been transported to other parts of the shell.

Eggs from the one-day age group were orientated on filter paper so that one-half (60) of them had the apex of the egg upwards and the other half had the apex downwards. Drops of an aqueous solution containing 0.2 per cent. of the TDNOC were placed on these eggs and they were then incubated at 75°F. and 70 per cent. R.H. Eighty-five per cent. mortality occurred in those on which the drops were placed on the apex and 83 per cent. mortality occurred when the drops were placed on the floor of the egg.

Using pure HETP in a similar manner, 100 per cent. mortality was obtained in both cases but 75 per cent. of the embryos did not develop when the drop was placed on the apex and 80 per cent. did not develop when the drop was placed on the floor of the egg. A pure sample of allethrin also caused 100 per cent. mortality when placed on the apex or on the floor of the egg. These results would seem to indicate that the insecticide did not necessarily have to penetrate through the micropyle to kill the embryo, although it is quite possible that liquids may more easily gain entrance to the embryo through the micropyle than through the general surface of the chorion.

While examining various staining reactions on whole eggs, a wide band of pits or canals was noticed extending horizontally around the circumference of the egg. Each pit was situated at the junction of a vertical and horizontal rib (fig. 16).

The external openings of these pits had a diameter of about 1 micron. The canals appeared to be filled with a material which had a slightly different refractive index from the rest of the shell. Leuckart (1855) and Verson (1893) both found somewhat similar tapering canals in eggs of various Lepidoptera and Wigglesworth and Beament (1950) have described and illustrated such canals in the eggs of *Bombyx mori* and *Ephesia kühniella*. They have shown by an injection technique with cobalt sulphide that these canals penetrate to the innermost layer of the chorion and believe that they have a respiratory function. From an inspection of the inner surface of the chorion of *D. oleracea* under the highest power of the microscope, the tapering ends of these canals can be seen but the layer of the shell in which they terminate is doubtful. An injection technique similar to that used by Wigglesworth and Beament (1950) would have to be undertaken before the terminating layer could be decided. These canals are present at all stages of development and it seems possible that they may be of considerable importance in the penetration of liquids through the chorion, especially as the experimental results seem to indicate that liquids do not necessarily enter only by the micropyle to kill the egg.

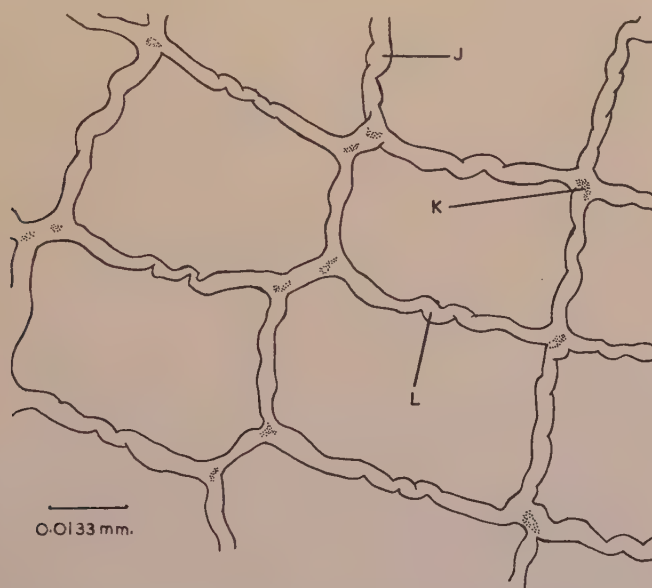


Fig. 16.—Ribbed structure of the egg-shell of *D. oleracea* showing the pit-like openings, K, at the junctions of the transverse (L) and the vertical (J) ribs.

All the penetration data obtained in this work are the result of dipping experiments in which the eggs were completely immersed in the test solution, thus ensuring that the liquid was in as close contact as possible with the whole external surface of the shell.

It has been mentioned previously when discussing the structure and properties of the egg-shell that the yolk material was not stained when the whole egg was immersed for several hours in solutions of various water-soluble stains but that the yolk became stained if the eggs had first been soaked in a wax solvent. This seemed to indicate that the water-proofing mechanism of the egg was the wax layer. The results of additional experiments in which groups of 1-day-old eggs were immersed for 12 hours in solutions having different properties indicated that distilled water, a saturated solution of sodium chloride in distilled water, and a

saturated solution of picric acid in distilled water did not apparently affect the embryonic material in any way, for when these eggs were removed from the solutions and incubated at 75°F. and 70 per cent. R.H. a very high proportion of them hatched. If eggs of a similar age, however, were immersed in basic fuchsin in 70 per cent. alcohol for a similar time the eggs became swollen and the yolks stained. It was of interest to note that in these eggs the vitelline-fertilisation membrane of some had become detached from the chorion, and the yolk, enclosed within the membrane, floated free within the shell. Presumably the alcohol, being a weak wax solvent, had dissolved the wax layer and freed the membrane which was attached to it. In addition, if groups of 1-day-old eggs were first soaked in acetone or xylol for a short time before being immersed in the aqueous solutions mentioned above they became swollen in distilled water and collapsed in saturated sodium chloride. A reason for this change in the permeability of the protective envelopes of the egg could be the disruption of the waterproofing wax layer by the wax solvents.

To determine if wax solvents would affect one age of egg more than another, the five ages of eggs as used in the spraying experiments (1-, 2-, 3-, 4- and 5-day-old eggs) were immersed for 30 minutes in varying concentrations of acetone. They were then removed, dried on filter paper and incubated at 75°F. and 70 per cent. R.H. The mortality data obtained is given in fig 17. Each point represents the result of a test on 25 eggs. It would appear that the youngest and the oldest eggs are affected to the greatest extent by the acetone and that the 3-day egg is affected the least. The order of resistance was $3 > 2 > 4 > 5 > 1$.

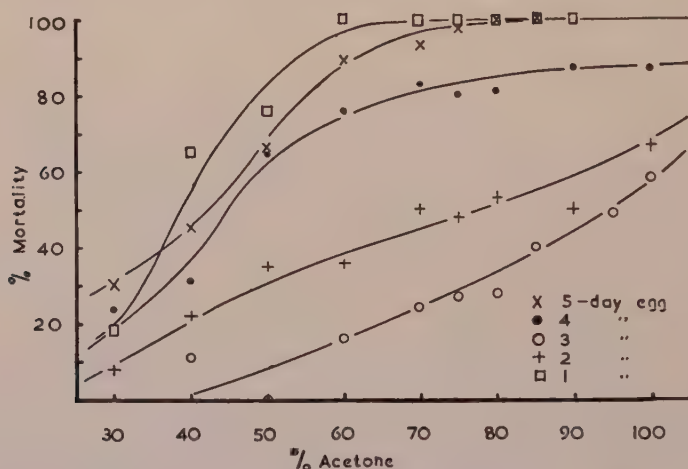


Fig. 17.—Dose/mortality curves for five ages of eggs of *D. oleracea* dipped for 30 minutes in various concentrations of acetone in water. After dipping, the eggs were incubated on filter paper at 75°F. and 70 per cent. relative humidity.

Mortality increased very quickly especially in the 1- and 5-day eggs as the acetone solution became more lipophilic and therefore became a more efficient wax solvent. Very much higher concentrations of acetone were required to give a 50 per cent. kill in the 2- and 3-day-old eggs than were required to give a similar kill in the 1- and 5-day and even the 4-day eggs. The difference in resistance among the 5 ages became less marked as the concentration of acetone became smaller and the solution more hydrophilic.

The results of an experiment using different concentrations of ethyl alcohol instead of acetone are given in fig. 18, where each point represents 50 eggs. The

order of resistance was $3 > 2 > 4 > 1 > 5$ although the differences between the 1- and 5-day eggs and between the 2- and 4-day eggs were very small.

Both the acetone and the ethyl alcohol affect the 1- and 5-day egg (an egg in which no apparent differentiation of the yolk has taken place and an egg in which the embryo has been almost fully developed) about equally. This would seem to indicate that the stage of development of the embryo does not affect its resistance to these materials. If this is so, then the differences in the concentration/mortality graphs of the five ages of eggs are dependent, to a greater extent, upon differences in the protective properties of the chorion and the membranes surrounding the yolk. A close correlation appears to exist between the ages of eggs in which the embryonic (sub-chorial) membranes are present and the ages which show the least susceptibility to the acetone and alcohol.

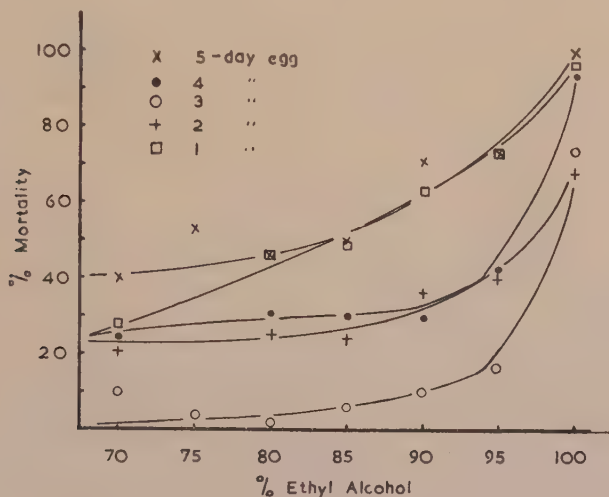


Fig. 18.—Dose/mortality curves for five ages of eggs of *D. oleracea* dipped for 30 minutes in various concentrations of ethyl alcohol in water. After dipping, the eggs were incubated on filter paper at 75°F. and 70 per cent. relative humidity.

It is of interest to note that concentrations of both the acetone and alcohol which caused a minimum of 100 per cent. mortality did not in all cases stop the development of the embryo in eggs which did not hatch. Many such eggs contained apparently fully developed larvae. A somewhat similar occurrence has been noted with the insecticides used in the spraying experiments.

Although the results of these dipping experiments with acetone and alcohol indicate that certain ages of eggs of *D. oleracea* are affected by these liquids to a greater extent than others and although such variations in resistance have been tentatively explained by variations in the numbers of embryonic membranes in the egg, it is realised that other factors may be having some effect on the apparent resistance found. The two factors which may be considered the most likely to affect resistance are the permeability of the egg-shell and related membranes and the susceptibility of the embryo. Since it is not at present possible to study changes in permeability of the egg-shell separately from changes in the susceptibility of the embryo, it does not seem possible to obtain direct information on the relative importance of differences in permeability of the egg-shell and differences in embryonic susceptibility at any given stage in the development of the egg but some information may be obtained by indirect methods.

A dipping experiment was carried out in which groups of eggs from the five age groups (1-, 2-, 3-, 4- and 5-day-old eggs incubated at 75°F.) were dipped for

varying lengths of time in a 0.1 per cent. concentration of TDNOC in an aqueous medium. This concentration of poison was sufficient to allow considerable hatching to take place in all ages of eggs. After dipping, the eggs from each age group were divided into two batches, A and B. The excess poison on batch A was removed on filter paper and these eggs were then incubated at 75°F. and approximately 70 per cent. R.H. The eggs in batch B were immediately washed in water for one minute, the excess liquid removed on filter paper and the eggs then incubated at the same temperature and humidity as batch A. In this way, presumably, a constant amount of poison was removed from the external surface of eggs of each age.

The numbers of eggs used, the dipping times and the mortality figures obtained are given in Tables XIV and XV. When considering the mortality figures obtained for each of the five ages of eggs at any one dipping time, it can be seen that the overall susceptibility of the egg is greatest at the beginning and end of the incubation period irrespective of whether the egg was or was not washed after treatment. This order of resistance appears to be $3 > 2 > 1 > 4 > 5$. This is a very similar resistance/age relationship to that found in the spraying experiments with allethrin and TDNOC at 57°F., and in the dipping experiments with acetone and alcohol.

TABLE XIV.

D. oleracea. Time/mortality data for eggs dipped in an aqueous solution containing 0.1% triethanolamine salt of 3:5 dinitro-ortho-cresol.

Batch A (excess poison removed on filter paper).

Age of eggs	1 day		2 days		3 days		4 days		5 days	
Time of dipping (mins.)	No. eggs	% mort.	No. eggs	% mort.	No. eggs	% mort.	No. eggs	% mort.	No. eggs	% mort.
10	54	49	77	45	49	31	56	58	83	71
20	32	56	48	49	73	49	57	74	83	96
30	75	70	55	66	80	60	56	85	77	100
40	86	79	66	69	53	70	30	100	82	100
50	75	89	70	85	54	73	35	100	78	100
60	84	92	61	87	59	85	29	100	73	100
70	81	98	60	100	60	87	40	100	61	100

After treatment the eggs were incubated at 75°F. and 70 per cent. R.H.

TABLE XV.

D. oleracea. Time/mortality data for eggs dipped in an aqueous solution containing 0.1% triethanolamine salt of 3:5 dinitro-ortho-cresol.

Batch B (washed in water after treatment).

Age of eggs	1 day		2 days		3 days		4 days		5 days	
Time of dipping (mins.)	No. eggs	% mort.	No. eggs	% mort.	No. eggs	% mort.	No. eggs	% mort.	No. eggs	% mort.
10	41	40	72	19	64	9	64	35	87	54
20	23	43	48	25	55	20	50	43	71	78
30	67	50	52	39	67	37	46	56	78	99
40	63	63	45	45	48	50	26	100	89	99
50	72	69	58	56	44	60	40	100	81	100
60	61	75	55	61	60	62	33	100	78	100
70	52	84	66	82	40	70	35	100	66	100

After treatment the eggs were incubated at 75°F. and 70 per cent. R.H.

A comparison of the mortality figures of batch B with those of a corresponding dipping time in batch A shows that mortality was reduced by about 20 per cent. (maximum variation 9–31 per cent.) in all ages of eggs irrespective of the immersion time if, of course, the immersion time had not been of sufficient length to cause a 100 per cent. kill. A 10-minute immersion in a 0.1 per cent. TDNOC solution, with subsequent removal of excess of insecticide with filter paper, produces mortality of from 30–70 per cent., dependent upon the age of the egg. It appears that penetration takes place very rapidly since, at immersion times of 10 minutes and over, a reduction in mortality of only 20 per cent. was obtained by removing the excess of insecticide by washing.

The reduction of mortality produced by washing the eggs remains approximately constant irrespective of the immersion time and the age of the egg; furthermore there appears to be a common resistance/age relationship both with the washed and the unwashed eggs for all the periods of immersion tested. If it be assumed, and the results of the respiration experiments which will be described later support this, that the insecticide is capable of penetrating the egg-shell and reaching the embryo in eggs of all ages, then it can be argued that the differences in susceptibility found with the eggs that were washed after treatment are due to differences in the rate of penetration of the egg-shell. These results agree well with what might be expected from the results of studies on the changes in structure and composition of the egg-shell during development.

While the rate of penetration is important during the immersion period, it is less likely to be important when considering the penetration of the insecticide coating left after the removal of the eggs from the dipping bath; the toxicity of the coating should depend on the amount of insecticide present providing the susceptibility of the embryo does not change. Since it may be assumed that a constant amount of insecticide was retained on the exterior of the egg after treatment and since this constant amount caused a constant increase in mortality, as compared with eggs from which the deposit was removed immediately after treatment, throughout the development period, the evidence is that the overall difference in the susceptibility of the eggs is due to changes in permeability of the egg-shell rather than to changes in embryonic susceptibility.

Respiration Experiments.

It has been pointed out previously that TDNOC can either inhibit embryonic development in all ages of eggs of *D. oleracea* or allow considerable development to take place depending upon the concentration applied. An attempt has been made to determine the effect of several concentrations of the poison on the oxygen consumption of the embryonic material in the 1-day egg and to determine if possible the speed of entry into the egg and the subsequent course of poisoning. It is known that 3:5 dinitro-ortho-cresol increases the rate of respiration and causes a rapid initial rise in oxygen uptake of adult *Tribolium castaneum* (Hbst.) (Lord, 1949, 1950), and Tattersfield (private communication) also found evidence that a somewhat similar increase in the respiration rate occurred in certain Lepidopterous eggs when treated with this poison.

In this experiment the respirometer described by Lord (1949) was used and it was found that 200 eggs gave a sufficiently high reading for slight deviations from the normal to be noticed. Three concentrations of TDNOC in an aqueous medium (5, 1 and 0.25 per cent.) were used because it was known that under the conditions of the spraying experiments the two higher concentrations would inhibit the development of the embryo but that the lower concentration would allow considerable development to take place.

Groups of 200 1-day-old eggs were dipped in the poison for 30 seconds, and care was taken that all eggs were thoroughly covered with the solution. They were then dried on filter paper and placed in the respirometer vessels. The

water bath was at a temperature of 75°F. The first readings were taken about one-half to one hour after the eggs had been put in the vessel. At this time the apparatus had obtained thermal equilibrium. The results obtained are given in fig. 19, and are expressed as microlitres of oxygen consumed by 200 eggs per hour.

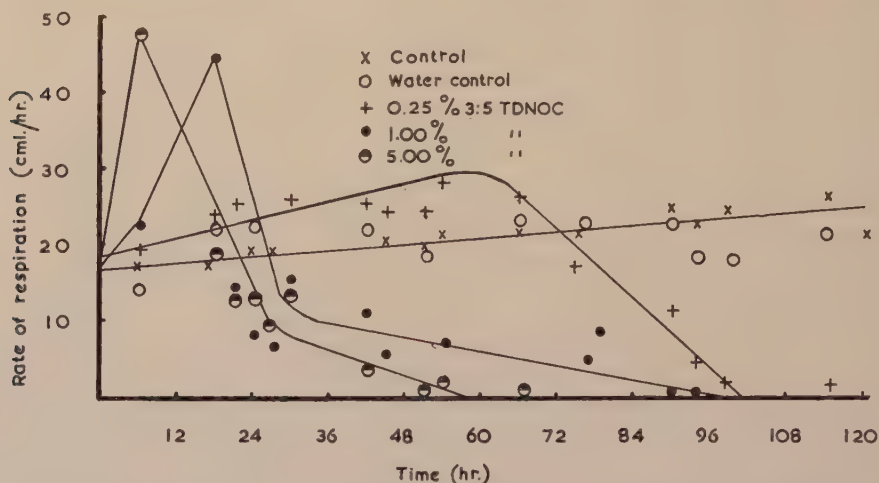


Fig. 19.—Effect of the concentration of TDNOC on the respiration of one-day-old eggs of *D. oleracea*.

The amount of oxygen consumed hourly by the controls increased steadily until hatching. The amount consumed hourly by the eggs treated with 0.25 per cent. TDNOC was initially slightly greater than that consumed by the controls, and it increased steadily for about 60 hours before a decline took place. When these eggs were dissected it was found that the embryo had developed apparently normally until it was approximately 3 days old and had then ceased development. It should be noted here that the slope of the respiration curve, especially in its initial stages, is not at all similar to the curve obtained in a later experiment (see fig. 23) with the same age of egg and when using a 0.2 per cent. TDNOC solution. The reason for this anomalous result is not known.

The 1 and 5 per cent. concentrations showed an increase in oxygen consumption as soon as it could be measured, and this rose to a maximum in 6 and 18 hours, respectively, after the eggs had been treated. After that the increase fell off rapidly especially in those eggs treated with the higher concentration. When these eggs were dissected no development of the embryo could be detected although the yolk membrane had begun to form in the eggs treated with the 1 per cent. concentration.

The TDNOC, regardless of its concentration, appeared to penetrate the chorion very quickly as judged by the responses of the embryonic material but the extent to which it exerted its toxic action depended upon the concentration used. The magnitude of the effect on the increase in oxygen uptake appears to be dependent upon the concentration of the TDNOC in the dipping solution. In addition, and this is important in considering the relationship between permeability of the egg-shell and embryonic susceptibility, this experiment provides evidence that, although the toxic material comes in contact with the embryo very shortly after treatment, development of the embryo continues if the concentration of the poison is not greatly in excess of the minimum required to give a 100 per cent. kill.

Another respiration experiment was carried out, the object of which was to determine if TDNOC entered each of the five ages of *D. oleracea* eggs at the same speed. If differences in the speed with which respiration was affected could be found in any one age, as compared with others, it would indicate, at least in part, that the ovicide was not entering all eggs at the same rate and therefore that one age of egg was more resistant to penetration of this liquid than another age. Two hundred eggs from each of the five age groups were dipped for 30 seconds in a 5 per cent. concentration of TDNOC in a sulphonated lorol-water medium. They were then dried on filter paper and put in the respirometer flasks. Readings taken one hour after the eggs had been dipped (30 minutes after the respirometers had become stabilised) indicated that eggs of all ages had become affected because the respiration rate of all had increased considerably over that of the controls. The oxygen uptake increased greatly for about three hours and then gradually decreased until about 24 hours after treatment, when it had decreased to nil. The time taken for a 5 per cent. concentration of the TDNOC to enter and affect the embryos in all ages of the eggs of *D. oleracea* was less than one hour, and thus the experiment failed in its original aim of showing differences in speed of penetration with age but it at least showed that rapid penetration occurred in eggs of all ages. Once again it appears that the poison reaches the embryo shortly after application.

One interesting fact that emerged from the toxicity experiments previously described and which has already been mentioned was that the development of the embryo proceeded to completion or near completion unless doses, greatly in excess of those required to give approximately 100 per cent. kill, were given. This occurred irrespective of the poison given or the time of application.

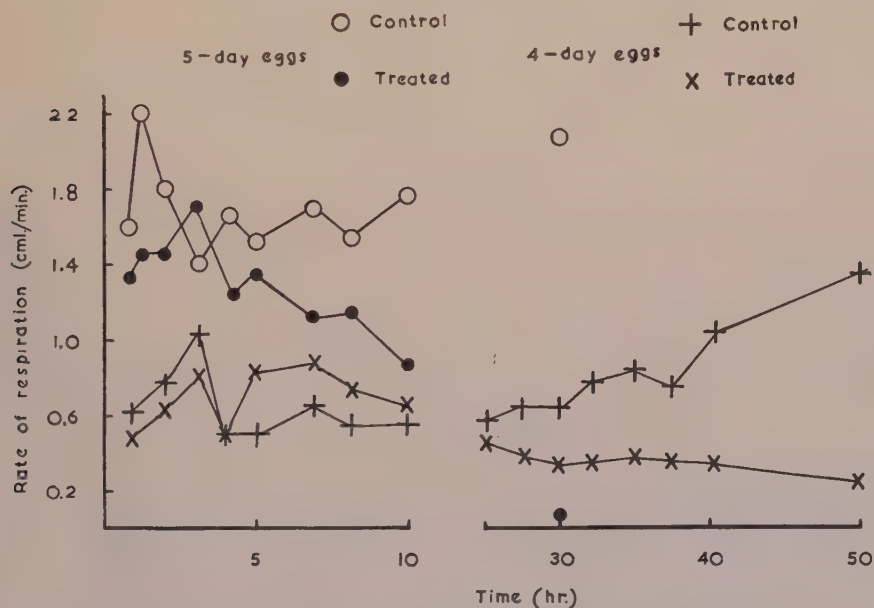


Fig. 20.—Effect of 0.2 per cent. concentration of TDNOC on the respiration of 5-day- and 4-day-old eggs of *D. oleracea*.

The respiration experiments just described show that the poison reaches and causes an effect on the embryo in a short time after application to the outside of the egg irrespective of the age of egg and the concentration of the insecticide applied. Because embryonic development proceeds until almost hatching time.

when a minimum LD100 is applied, the indications are that the end of the incubation period is a most critical time in the development of the embryo. It seems possible, however, that even although morphological changes continue to take place in the embryo, physiological changes which could ensure the ultimate death of the embryo might be produced at different stages in the incubation period.

It appeared that a study of the respiration curves of eggs of different ages treated with an approximate LD100 of TDNOC might give some information on the course of poisoning which could not be obtained from an inspection of the morphological changes occurring and might supply additional information on the relative importance of permeability of the egg-shell and embryological susceptibility in determining the overall toxicity of the poison.

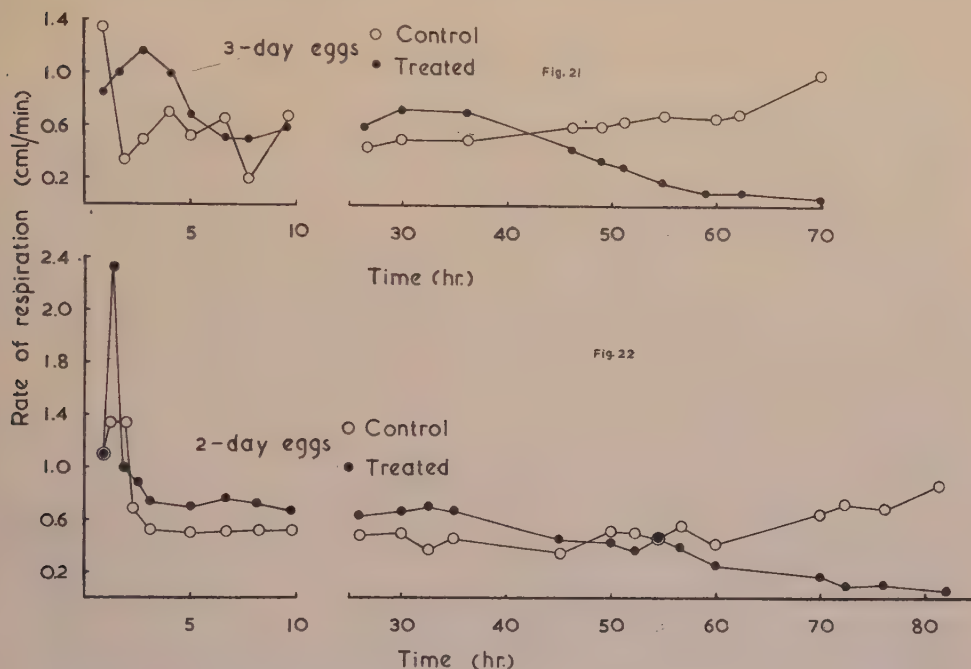
A respiration experiment was therefore carried out at 75°F., in which the 5 ages of eggs of *D. oleracea* were treated with a 0.2 concentration of TDNOC in a sulphonated lorol-water medium. Three hundred eggs from each of the age groups were dipped for 30 seconds in the poison, the excess liquid was drawn off on filter paper and the eggs placed in the respirometer flasks. The initial readings were taken one hour after the treatment of the eggs. A control in which the eggs were dipped for the same length of time (30 secs.) in a 0.1 per cent. solution of sulphonated lorol in water was carried out for each age group.

From fig. 20 it can be seen that there was a sharp initial rise in the oxygen consumption of the 5-day eggs which reached its maximum about three hours after the application of the poison. This initial rise was followed by a sharp decline within the next 30 minutes below the respiration rate of the control and which continued as a more gentle decline until the control eggs had hatched. Thus the rate of penetration of the poison and the speed with which it affected the embryo were very rapid. From a knowledge of the structure of the egg-shell at this stage of egg development, it seems possible that this speed of penetration could be a result of the resorption of the embryonic membranes by the embryo at this time, thus removing some of the obstacles through which the poison would have to penetrate before reaching the embryonic material. A 100 per cent. kill occurred but a few attempts at hatching had taken place indicating that development of the embryo had continued after treatment. However, it can be inferred that the poison had caused a sufficient physiological upset to ensure death even although the usual morphological changes in the embryo appeared to take place. Considerable fluctuation occurred in the controls with this and other ages of eggs during the first two hours, after which time consistent readings were obtained. No specific reason can, as yet, be given for this fluctuation.

The respiration rate of the 4-day eggs (fig. 20) did not increase as rapidly as in the 5-day egg and, in addition, this increased respiratory rate fell off more slowly, and remained above the control rate from the fifth until at least the tenth hour after treatment, after which it fell below the control. Thus the rate of penetration of the poison appeared to be slower in this age of egg than in the 5-day eggs. The embryos in the treated 4-day-old eggs had developed apparently normally, as judged by the morphological characteristics, until almost hatching time but had made no noticeable attempts to hatch. Once again, it can be inferred that the poison had in some way upset some physiological process shortly after application, but that this upset did not have an apparent effect on the morphological development of the embryo until shortly before hatching. However, the decrease in the rate of insecticide penetration in the 4-day egg can be correlated with the presence of embryonic membranes at that time. In addition this correlation can be carried further because the first definite morphological evidence of an effect on the embryo appears at the time when the embryonic membranes are beginning to be resorbed by the embryo. This resorption must be a gradual process throughout the 5th day, and the amount of any extra poison which may come in contact with the embryo at this time would presumably be

small, and this may account for the slow decrease in oxygen consumption in the eggs treated on the fourth day.

The increase in the oxygen consumption of the 3-day egg (fig. 21) was a more gradual process than in the 5-day egg and, as in the 4-day egg, the increased respiration rate fell off more gradually to the level of the control. However, the respiration rate of the treated and the control eggs remained much the same throughout the 4th day of incubation, but between the 4th and 5th day (42 hours after treatment) the respiration rate of the treated eggs fell below the control and continued falling until the controls had hatched. By dissection it was noted that, morphologically at least, the embryos in the treated eggs had developed to almost the 5th-day stage. The slower and smaller initial effect of the poison on the embryo can be correlated with the presence of the embryonic membranes in this age of egg which may hinder the penetration of the liquid.



Figs. 21 and 22.—Effect of a 0.2 per cent. concentration of TDNOC on the respiration of 3-day and 2-day-old eggs of *D. oleracea*.

In both the 3- and 4-day eggs, the ultimate effect of the poison on the embryonic material becomes apparent between the 4th and 5th day of incubation. Thus, this period in the development of the embryo appears to be a critical one either because of the beginning of the resorption of the embryonic membranes or because of the greater susceptibility of the embryo itself at that time.

The effect of the TDNOC on the oxygen consumption of the 1-day egg and the 2-day egg was much the same (figs. 22 and 23). This effect consisted of a very sharp initial rise in oxygen consumption followed by a sharp decrease to the control level within about 2 or 3 hours after treatment. As in the 5-day egg, the rate of penetration was very rapid, and could be correlated with the absence or partial absence of embryonic membranes in these ages of eggs. The respiration rate of the 1- and 2-day eggs, however, remained much the same as the controls until between the 4th and 5th day of incubation when respiration in both

these age groups fell below that of their respective controls and continued to decrease until the controls hatched. The embryos in these treated eggs had proceeded apparently normally to almost the 5th-day stage. Thus, although the poison entered and affected the embryonic material very shortly after application,

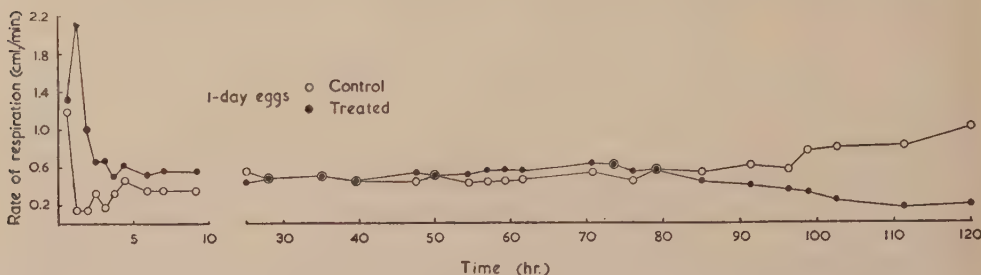


Fig. 23.—Effect of a 0.2 per cent. concentration of TDNOC on the respiration of 1-day-old eggs of *D. oleracea*.

the amount which entered was not sufficient to prevent further embryonic development despite the fact that it was undoubtedly interfering with physiological processes. Once again the stage of development between the 4th and 5th day of incubation appeared to be the most critical.

Although the shape of the respiration curves indicated that penetration of the poison takes place more rapidly in the 5-day, 2-day and 1-day egg than in the other two ages, its ultimate effect on the embryological material is much the same. In all cases a physiological upset was produced after the application of the poison which, while it did not prevent further morphological differentiation of the embryo, was sufficient to ensure that the embryo would ultimately die shortly before emergence.

Although a correlation appears to exist between the presence of the embryonic membranes and the rate of penetration of the poison, the final effect on the embryo indicates that, regardless of the time of application of the poison, there is a certain stage in development when the embryo is more likely to succumb to the effects of the poison. Until this time the embryo, judged on morphological evidence, appeared normal. Such an occurrence would appear to be dependent upon the concentration of insecticide used, because embryonic development proceeds to completion and attempts at hatching take place in eggs of all ages when the concentration of insecticide gives only about 50 per cent. kill, whereas development of the embryos is immediately inhibited in eggs of all ages if the concentration of the poison used is greatly in excess of the LD100.

The experimental evidence in this series of respiration tests suggests that the changes in resistance throughout the development of the egg are primarily due to the changes in the permeability of the egg-shell rather than to changes in the susceptibility of the embryo. In addition, this evidence on changes in permeability with age is what might be expected from information obtained in the study carried out on the structure and composition of the egg-shell.

These experiments also provide evidence that the poison penetrates the egg-shell and reaches the embryo in a few minutes after treatment irrespective of the age of the egg. Physiological disturbances are produced but external morphological differentiation proceeds to completion or near completion unless dosages far in excess of those required to give approximately 100 per cent. kill are administered. Under these conditions and with any particular age of egg, the shape of the respiration curve will vary with the concentration of poison applied but cessation of development apparently always occurs at the same time. Thus it may be inferred that this particular stage of embryonic development is critical.

Discussion.

Previous work on the subject of changes of resistance of insect eggs with age have been concerned chiefly with the demonstration that where a particular insecticide is used on a particular species of egg, changes of resistance occur during development. In some of the work an estimate of the magnitude of these differences has been obtained.

Spraying experiments.

In the spraying experiments described here, the effect of a number of insecticides on a number of species of insect eggs were examined under comparable conditions. This was done not only to determine whether changes in resistance occurred and, if present, to estimate their magnitude, but chiefly to try to determine some of the major factors influencing these changes in resistance.

As a result of these experiments it appears that either the magnitude, or the pattern, or both the magnitude and the pattern, of the change in resistance of insect eggs to insecticides with age can be influenced by (1) the nature of the insecticide; (2) the species of insect egg; (3) the incubation conditions of the eggs.

(1) *The insecticide.* With any given species of egg and set of experimental conditions the magnitude of the differences in resistance that occur during development will alter with the insecticide. For example, the change in the

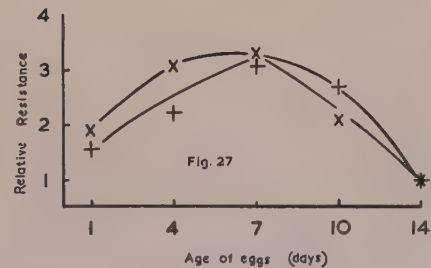
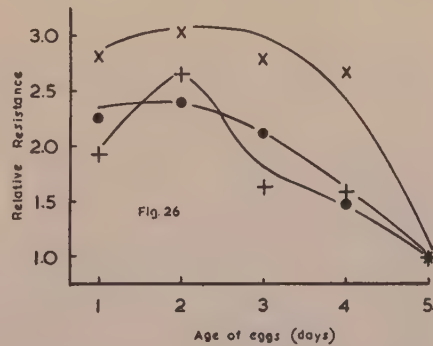
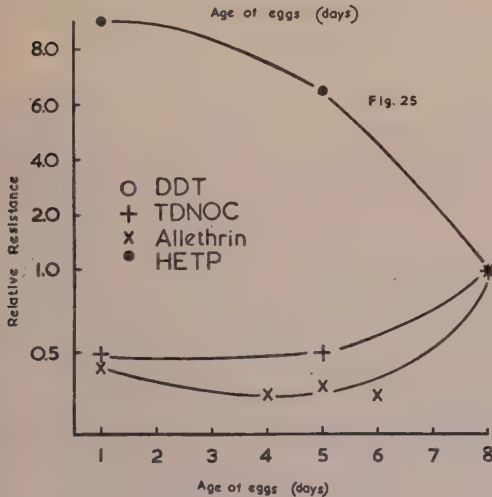
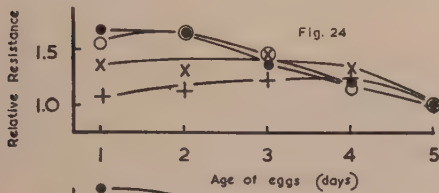


Fig. 24.—Relative resistance/age curves determined for eggs of *D. oleracea* in the spraying tests at 75°F.

Fig. 25.—Relative resistance/age curves determined for eggs of *D. fasciatus* in the spraying tests at 75°F.

Fig. 26.—Relative resistance/age curves determined for eggs of *E. kuhniella* in the spraying tests at 75°F.

Fig. 27.—Relative resistance/age curves determined for eggs of *D. oleracea* in the spraying tests at 57°F.

resistance of eggs of *D. fasciatus* to both TDNOC and allethrin was approximately $3\times$ while it was approximately $10\times$ with HETP under the same set of experimental conditions.

Where relatively stable insecticides were used, the patterns of resistance changes for a given species of egg tended to be similar. Thus, although there were differences, the pattern of resistance for TDNOC appeared much the same as that for allethrin when tested on *Diataraxia oleracea*, *Ephestia kühniella* and *Dysdercus fasciatus* (figs. 24, 25, 26), although the pattern obtained on *D. fasciatus* differed from that obtained on the two Lepidoptera. With HETP, however, the pattern of resistance may differ considerably from the two previously mentioned insecticides. This poison always increased in effectiveness as the age of the egg increased, irrespective of the species of the egg (figs. 24, 25, 26).

The reasons for both the differences and similarities found in these experiments can at present be only a matter of speculation, but they could be explained on a basis of changes in the permeability of the chorion and of the stability of the insecticides. The further experimental data obtained on chorion structure and permeability discussed in the succeeding sections at least do not conflict with this explanation. Differences in the magnitude of changes of susceptibility with a given species of egg that occur with different insecticides may be due to differences in the permeability of the chorion that occur during development, affecting the penetration of one insecticide to a greater extent than another. The data obtained in this series of experiments are inadequate on this point since only small differences occurred between TDNOC and allethrin, and the larger differences that occur between HETP and TDNOC and allethrin are explained in the following section on the basis of the relative ease of hydrolysis of the HETP. Differences in the pattern of susceptibility between a relatively stable insecticide such as TDNOC and one that is very unstable, such as HETP which is very easily hydrolysed, may be because the insecticide not only has to penetrate the chorion but may also remain *in situ* for some time and take effect at a critical stage of development.

With TDNOC the predominating factor would be penetration, hence its effectiveness might depend to a considerable extent on the permeability of the chorion at the time of application. With HETP on the other hand the time interval between application and the poison taking effect may be more important than differences in chorion permeability, since if this interval is long the poison will be hydrolysed and rendered ineffective.

(2) *The species of egg.* It appears that under a given set of conditions and with a given insecticide both the magnitude and the pattern of resistance can vary with the species of egg. For instance, at 75°F. using TDNOC, only slight changes of resistance during development were found with eggs of *Diataraxia oleracea*, but these changes were more marked with eggs of *Ephestia kühniella*, although the pattern of change is similar. However, when eggs of *Dysdercus fasciatus* were used as test subjects, the maximum difference in resistance found was greater than in either of the other two species and the pattern of the change is quite different.

At 75°F. the eggs of both *D. oleracea* and *E. kühniella* complete their development in about 5 days (and being both Lepidoptera they develop and hatch in a similar manner). This can account for the similar pattern of resistance change with the two species. The difference in the magnitude of the change of resistance in the two species could be explained either by greater differences in chorion permeability or of embryonic susceptibility or a combination of both occurring during development with *E. kühniella* than with *D. oleracea*. No experimental data is available on the relative importance of permeability and embryonic susceptibility. The differences in both magnitude and pattern of the changes in

resistance of eggs of the bug *Dysdercus fasciatus* from those of the two Lepidoptera can reasonably be explained by the differences that are likely to be present between Lepidoptera and Hemiptera in the changes in chorion structure during development and in egg hatching procedure. Here again however no detailed experimental study can be put forward in support of this hypothesis.

(3) *Incubation temperature.* The earlier experiments with eggs of *Diataraxia oleracea* incubated at 75°F. showed only small differences in resistance to occur throughout development. With the four insecticides tested these differences were: HETP, 1:1.80; DDT, 1:1.57; allethrin, 1:1.45; and TDNOC, 1:1.18. However, when the incubation temperature was lowered to 57°F. and the effects of allethrin and TDNOC were again examined the differences in resistance were found to have increased, the figures being 1:3.26 for allethrin and 1:3.48 for TDNOC. The pattern of change in resistance remained substantially unchanged. There were, however, some differences between the action of the two poisons at the two incubation temperatures. With both poisons the youngest and the oldest eggs remained the most susceptible with resistance increasing up to half way through incubation and then decreasing. However, the lower incubation temperature decreased the resistance of all ages of eggs to allethrin, although it affected the young and the old eggs to the greater extent; with TDNOC there was practically no change in the resistance of the middle-aged eggs, but the lower temperature decreased the resistance of the young and the old eggs.

No explanation which has any supporting experimental evidence can be put forward to account for the observed effects of incubation temperature. Since, however, it may be of considerable practical importance, further information would appear to be highly desirable.

It was hoped that the observations on the effect of the various insecticides on embryonic development, most of which were made on *D. oleracea*, might give some indication of the mode of action of the poison, in particular if a given insecticide was most effective on a specific stage of development of the embryo. However, no definite information on this point was obtained and there was only the indication that with all the insecticides tested the fully developed embryo was the critical, if not the most susceptible, stage.

Thus as the concentration of insecticide was decreased the percentage of dead eggs containing fully developed embryos increased, irrespective of the chemical nature of the insecticide. It may be inferred from this that if an egg received a dose of poison just sufficient to kill it, development proceeded until the embryo is fully formed, when the poison takes effect. If the dose of poison is increased above the minimum lethal dose to a sufficient extent, all the poisons except DDT appear able to inhibit embryonic development, although the ratio between the minimum lethal dose and that required to inhibit development varies greatly with the poison. Thus 0.7 per cent. to 1 per cent. of TDNOC was about the minimum required for 100 per cent. kill of eggs and 1 per cent. to 2 per cent. would inhibit embryonic development; with allethrin, 0.1 per cent. to 0.5 per cent. would give 100 per cent. kill but over 5 per cent. was required to inhibit embryonic development; while with HETP 1.25 per cent. will give 100 per cent. kill but the undiluted material is required for inhibition of development. Potter and Tattersfield (1943) using a wider range of insecticides and species of egg have observed that embryonic development may proceed after treatment with a number of insecticides, but that eggs killed by 3:5 dinitro-o-cresol were normally undeveloped. They further showed that the presence of petroleum oil in the medium of application prevented development.

It seems quite impossible with spraying experiments of this type to decide whether any particular stage of embryonic development is more susceptible than

another to any given poison; one cannot assess the effect of the chorion in governing the amount of poison actually reaching the embryo, nor how this changes during development. For instance, since it appears that with the minimum lethal dose of any of the insecticides tested, embryonic development proceeds to completion or near completion before death occurs, it could be inferred that the fully developed embryo is the most susceptible stage to all the insecticides tested. However it is known that prior to hatching the internal membranes of the chorion are broken down and resorbed together with the serosal fluid. It may be that these processes result in poison being released from the chorion and taken up by the nearly fully developed embryo in amounts sufficient to kill it. Further information on these points was obtained in the respiration experiments and is discussed later.

It appears therefore that the most that can be inferred with any certainty is that, with the exception of DDT, all the insecticides tested, *viz.*, TDNOC, allethrin and HETP, are capable of killing the embryo in the initial stages of development providing sufficient poison reaches it. The failure of DDT to inhibit development at the highest concentration that was applied, which was in the order of 50 times the dosage necessary to give 100 per cent. kill, might still be because insufficient DDT reached the embryo in its early stages, even at these high dosages.

The observation that the appearance of eggs killed at an early stage of development differed with the insecticide and appeared characteristic for each insecticide is of interest because it provides visual evidence that their mode of action is different. Unfortunately the appearance of the dead eggs provides no obvious clue to the biochemical system on which the poison operates.

Chorion structure.

The differences in susceptibility which have been found to occur during the development of the egg could be caused by one of at least two factors or a combination of these. These factors are:—

- (1) Changes in the inherent susceptibility of the embryonic material.
- (2) Changes in the permeability of the egg-shell and embryonic (sub-chorial) membranes.

In addition, such factors as the absorption of the extra-embryonic fluids and the ingestion of the embryonic membranes which may contain accumulated insecticides must also be considered.

In order to provide some explanation, which could be based on experimental data rather than on pure hypothesis, for the differences in susceptibility that were found in the spraying experiments, a study was made of the structure and composition of the shell in the egg of *D. oleracea*. Special attention was paid to the shell structure in the five ages of eggs used in the spraying tests at 75°F. In addition, experiments were carried out to determine the ease of penetration through this egg-shell.

It was shown that at certain times during the development of the egg, membranes were formed which to some extent may hinder the penetration of insecticides to the embryo. Just prior to oviposition the chorion of the egg-shell consists of at least five layers:—

- (1) The exochorion, a fairly thin layer on the external surface of the shell which appears to be composed of lipoproteins.
- (2) A soft protein layer approximately 2 microns thick.
- (3) The resistant endochorion, a fairly thin (0.5 microns) proteinaceous layer, the internal surface of which is very hydrophobic.
- (4) A "waterproofing" layer composed of wax-like material.
- (5) A vitelline membrane.

The properties of the chorion appear to remain relatively constant throughout the

life of the egg. Immediately after oviposition, the vitelline membrane is modified to become the vitelline-fertilisation membrane, and a cement layer is added to the external surface of the egg. During embryonic development, embryonic or sub-chorial membranes are formed by the oöcyte and are separated from the vitelline-fertilisation membrane by a serosal fluid. Both the embryonic membrane (serosa) and the serosal fluid reach their maximum development in the middle of the incubation period. At this time, the embryo enclosed within the serosal membrane can be removed intact from the shell and when incubated under optimum conditions of temperature and humidity it continues its development, and a normal live larva is produced. Between the 4th and 5th day of incubation at 75°F., the serosal membranes are slowly resorbed by the embryo. If HETP be excluded as a special case owing to its ease of hydrolysis, the periods of maximum susceptibility of the eggs of this species to insecticides corresponds very closely to the periods during incubation before the embryonic membranes are formed and the period when they are being resorbed or are resorbed, i.e., to the beginning and end of the incubation period.

A number of experiments were carried out which were designed to give information on the factors influencing the permeability of the egg-shell of *D. oleracea*.

As with the spraying experiments, these experiments on the permeability of the egg-shell are unsatisfactory, because the death of the egg was frequently the criterion of penetration and no method has been found of separating changes in mortality due to changes in permeability from those due to changes in embryonic susceptibility.

It is important to find out if changes in resistance are caused by changes in specialised areas of the egg-shell rather than in the layers of which it is composed. Thus Beament (1948a, b) showed that with a number of substances penetration through the chorion of the egg of *Rhodnius prolixus* Stål. occurred almost entirely through the micropylar canals, and his experiments were designed to show that this fact greatly influenced penetration and ovicidal effects.

At least with the egg of *D. oleracea* the micropyles do not appear to be of such great importance in governing penetration.

The experiments where measured drops of TDNOC, HETP and allethrin in distilled water were placed on specified areas of egg-shell indicate that these insecticides can penetrate the general chorion and that they do not of necessity penetrate via special routes such as the micropylar or respiratory canals (Wigglesworth & Beament, 1950). In fact, no differences were found when the micropylar area and an unspecialised area were compared. However, the experiments are open to criticism, for one cannot be sure the poisons did not reach and enter by the canals, even when placed at sites remote from them.

The experiments using aqueous solutions of sodium chloride and sodium picrate show that the intact egg-shell of *D. oleracea* is impermeable to water and the evidence points towards its impermeability to the solutes as well. From these experiments and the others included in this study, no definite statement can be made on the specific chemical and physical properties governing the penetration of solutes from aqueous media. Ionisation may be important; thus molecules such as sodium chloride and sodium picrate, which ionise, do not penetrate, but an unionised molecule such as HETP does. HETP may also be able to penetrate as a result of its solubility in both water and lipids.

The experiments show that fat solvents and the solutes contained in them can penetrate the egg-shell of *D. oleracea*. Thus it appears that both alcohol and the basic fuchsin dissolved in it readily penetrate the chorion. Finally it appears that pretreatment with a fat solvent renders the egg-shells permeable to water. It is, however, apparently still impermeable to molecules such as sodium chloride, as shown by the collapsing of eggs immersed in saturated

solutions of sodium chloride. The conclusions are that the impermeability of the egg-shell to water is due to the presence of lipoid material which can be removed by fat solvents, and that no precise statement can be made on the chemical and physical properties influencing the penetration of other molecules. Factors such as capacity to ionise and thus carry a charge, fat solubility, and the number of layers present on the egg-shell, appear to be important.

Some evidence on the influence of additional layers on penetration is given by the experiments on the toxicity of aqueous solutions of acetone and alcohol, where it was shown that the eggs were most resistant during the middle period of development when the egg-shell consisted of the maximum number of layers. The differences in resistance obtained in these experiments could be due to differences in embryonic susceptibility rather than chorion permeability but in the present state of knowledge it does not seem probable that the undeveloped embryo and the fully developed embryo would be the two least resistant stages with the middle stages more resistant, particularly since the spraying experiments give a similar pattern with other poisons of widely different chemical and physical characteristics. The experiments where eggs of different age groups were immersed for various lengths of time in an aqueous solution of TDNOC provide additional evidence that changes of embryonic susceptibility are not the governing factor influencing the changes in resistance. An approximately constant difference of 20 per cent. kill between washed and unwashed eggs indicates that throughout all stages of development a constant amount of poison left on the egg-shell gave approximately the same increase in kill, the changes in resistance that were found in both washed and unwashed eggs apparently being due to differences in rate of penetration from aqueous solution. This experiment indicated that penetration of TDNOC from aqueous solution was rapid, and this point was further studied and compared in the respiration experiments.

Respiration experiments.

The respiration experiments were carried out entirely with the water soluble TDNOC and the eggs of *D. oleracea*, and some of the results may not have any general significance.

The experiments showed that the poison reached the embryo and caused a rise in respiration rate within the period required to equilibrate the apparatus, *i.e.*, $\frac{1}{2}$ –1 hour. This appeared to take place irrespective of the age of the eggs, and showed that at least a proportion of the poison was reaching the embryo soon after application.

The shape of the respiration curves depended on the concentration of poison applied and the age of the egg, but, with one anomalous exception (see p. 564) the respiration rose to a maximum and fell to the control level within five hours, irrespective of the age of the egg. Except where concentrations greatly in excess of the minimum LD100 were applied, respiration would then continue apparently normally until 25 to 40 hours before hatching when it fell below the level of the control. With concentrations greatly in excess of the minimum LD100, there is, after the initial rise, a continuous drop in respiration until death, which may be at any stage of development.

Two possibilities must be considered in attempting to explain these results. First, only a small proportion of the poison reaches the embryo soon after application and the lethal dose reaches it when the serosal membrane is broken down towards the end of the incubation period; a similar suggestion has been made by Beament (1948b). The embryo may then vary in susceptibility throughout the period and the toxicity will depend on the amount of poison entering the egg-shell at the time of application. The fact that doses considerably in excess of the minimum LD100 can kill eggs in an early stage of development may be because, with very high doses, even the small proportion of the dose that reaches the

embryo is sufficient to kill it. Secondly, it is possible that a high proportion or all of the applied poison may reach the embryo soon after application, but only takes effect at the end of the incubation period. If this is so, the fully developed embryo may be said to be the most susceptible stage. Here again the toxicity will depend on the amount of poison entering the egg-shell at the time of application. In this case the period of normal respiration and apparently normal development that follows the rise and fall of respiration after the application of a minimum lethal dose may be because a metabolic disturbance is produced and a system that is only vital to the fully developed embryo is inactivated, development then proceeds apparently normally until near completion or completion, when the absence of the vital system causes death. The observation by Potter and Tattersfield (1943) that embryonic development proceeded in lepidopterous eggs treated with rotenone and derris resins but that development is abnormal since the eggs remain filled with fluid even in the final stage of the embryo might be used to support this theory. In this case the interference with the vital system was such as to cause visible symptoms of abnormality in the egg although the embryo itself continued to develop. The effect of high doses may be because high concentrations have a secondary effect on other systems which are necessary for more generalised metabolism.

It seems that, irrespective of the proportion of the poison that reaches the embryo soon after application, the critical factor determining ultimate toxicity is the rate of penetration into the egg-shell. The differing shapes of the respiration curves in the experiments where 0.2 per cent. TDNOC was applied to eggs of different ages (figs. 20-23) indicate that the rate of penetration differs with the age of the egg and these differences can be correlated with the variations in the number of layers in the egg shell, just as in the spraying experiments and in the experiments on permeability. The respiration experiments therefore also suggest that the differences in resistance with age that have been found are primarily due to the differences that occur in the permeability of the chorion.

Summary.

Laboratory spraying experiments were carried out with DDT, allethrin, the triethanolamine salt of 3:5 dinitroresol (TDNOC) and HETP against eggs of different ages of *Diataraxia oleracea* (Lepidoptera) and with allethrin, HETP and TDNOC against eggs of different ages of *Ephestia kühniella* (Lepidoptera) and *Dysdercus fasciatus* (Hemiptera) under controlled temperature and humidity conditions.

The shape of the resistance/age curves obtained varied with:

- (1) the incubation temperature.
- (2) the species of egg.
- (3) the insecticide.

In eggs of *Diataraxia oleracea* very small differences in susceptibility with age were found with any of the insecticides at 75°F. and 60-70 per cent. R.H., but definite resistance/age curves were obtained with allethrin and TDNOC when the eggs tested had been incubated at 57°F. In this case the youngest (1-day) and the oldest eggs (14-day) tested were the least resistant. DDT and HETP were only tested against the eggs of *D. oleracea* incubated at 75°F.; only small differences were found at this temperature. There were indications that the one-day-old eggs were the most resistant to these insecticides, but the results were barely significant.

The shape of the resistance/age curves for the two species of Lepidoptera tested under similar experimental conditions varied slightly but had a general similarity, but those for the Hemipteran eggs were very different. This is ascribed to differences in chorion structure and process of development between these two species of eggs.

With any one species of egg, the shape of the resistance/age curve differed from one insecticide to another. Usually, however, the shapes of the curves obtained for allethrin and TDNOC were much the same. The different type of curve with HETP is ascribed to its ease of hydrolysis.

It was observed that embryonic development continued after the application of any one of the four poisons to eggs of *D. oleracea*, unless the concentration of the insecticide was considerably in excess of the minimum required to give a 100 per cent. kill. There was some indication that the ability to inhibit embryonic development varied with the insecticide. The data indicated that whereas only two to three times the minimum LD₁₀₀ of TDNOC is required to inhibit development, the figure for allethrin and HETP is in the order of 50 to 100 times. The evidence available indicates that DDT does not inhibit embryonic development.

The appearance of eggs killed at an early stage in their development was characteristic for each insecticide. When development is inhibited by TDNOC the eggs turn brown and several small brown circles, which appear to be composed of yolk material, become closely applied to the chorion; with allethrin the yolk contents become dark in colour and quite liquid; with HETP the yolk contents are colourless and the chorion opaque.

From a study of the structure and composition of the protective envelopes in different ages of eggs of *D. oleracea* it has been found that membranes are formed by the ovum during development which may hinder the penetration of insecticides to the embryo. Maximal development of these membranes occurs near the middle of the incubation period. It was possible at this time to remove the embryo enclosed within these membranes from the chorion without apparent injury to the embryo itself.

Just prior to oviposition the egg-shell consists of at least five different layers:

- (1) The exochorion, a fairly thin layer on the external surface of the shell which appears to be composed of lipoproteins.
- (2) A soft protein layer approximately 2 microns thick.
- (3) The resistant endochorion, a fairly thin (0.5 microns) proteinaceous layer, the internal surface of which is very hydrophobic.
- (4) A "waterproofing" layer composed of wax-like material.
- (5) A vitelline membrane.

After fertilisation and oviposition, changes take place in the vitelline membrane and the resultant membrane has been called the "vitelline-fertilisation membrane." A cement layer is deposited on the chorion by the female during oviposition but otherwise the chorion in the laid egg is apparently unchanged from that of the unlaid egg from the oviduct.

The results of dipping and washing experiments with eggs of *D. oleracea* indicated that the resistance/age relationships found was due to a difference in the ability of the insecticide to penetrate the shell layers and membranes rather than to differences in the inherent susceptibility of the embryos of different ages.

Considerable embryonic development took place after the application of the poison to all ages of eggs. Observations showed that there were two stages during embryonic development when the death of the embryo was most likely to occur if the concentration of poison did not immediately inhibit further development. When death occurred at the first critical stage, the embryo developed to completion or near completion but no visible attempts to hatch occurred. When it occurred at the second critical stage, holes in the shell were visible where hatching had been attempted.

The speed of penetration of TDNOC into eggs of *D. oleracea* as determined in respiration experiments was very rapid irrespective of the age of the egg. The poisoned eggs showed a marked initial rise in oxygen consumption, the extent of which depended upon the age of the egg, a greater increase being noticed

in the 1-2- and 5-day eggs than in the 3- and 4-day eggs. Although the metabolism of these eggs was affected, the embryos continued their development apparently normally until reaching one of the two critical stages. It is suggested that the number of embryos that succeed in passing the critical stages and in hatching depends upon the extent of the initial metabolic disturbance caused by the poison which in turn depends upon the amount of poison reaching the embryonic material. The extent of the metabolic disturbance is an index of the amount of poison entering the egg-shell and therefore of ultimate toxicity. The shape of the respiration curves indicate that the majority of the poison reaches the embryo either soon after application or towards the end of the incubation period when the serosal membrane and fluids are resorbed. Thus differences in resistance that occur in the eggs of different ages are due to changes in the permeability of the egg-shell which allows more or less poison to reach the embryo.

A close correlation appears to exist between the age of egg which shows maximum resistance to the insecticide and the age of egg in which the embryonic membranes are present at their maximum stage of development.

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References.

- BEAMENT, J. W. L. (1945). The cuticular lipoids of insects.—*J. exp. Biol.*, **21**, pp. 115–131.
- BEAMENT, J. W. L. (1946a). The formation and structure of the chorion of the egg in an Hemipteran, *Rhodnius prolixus*.—*Quart. J. micr. Sci.*, **87**, pp. 393–439.
- BEAMENT, J. W. L. (1946b). The waterproofing process of eggs of *Rhodnius prolixus* Stål.—*Proc. roy. Soc., (B)* **133**, pp. 407–418.
- BEAMENT, J. W. L. (1948). The penetration of the insect egg-shells. I. Penetration of the chorion of *Rhodnius prolixus*, Stål.—*Bull. ent. Res.*, **39**, pp. 359–383.
- BEAMENT, J. W. L. (1949). The penetration of insect egg-shells. II. The properties and permeability of sub-chorial membranes during development of *Rhodnius prolixus*, Stål.—*Bull. ent. Res.*, **39**, pp. 467–488.
- BEAMENT, J. W. L. (1951). The structure and formation of the egg of the fruit tree red spider mite, *Metatetranychus ulmi* Koch.—*Ann. appl. Biol.*, **38**, pp. 1–24.
- BLICKLE, R. L. (1942). Penetration of oils into insect eggs. (a) Influence of oil characteristics. (b) Influence of age of egg and of species. Studies of contact insecticides XVI.—*Tech. Bull. N. H. agric. Exp. Sta.*, no. 79, 14 pp.
- CAMPBELL, F. L. (1929). The detection and estimation of insect chitin; and the irrelation of "chitinization" to hardness and pigmentation of the cuticula of the American cockroach, *Periplaneta americana* L.—*Ann. ent. Soc. Amer.*, **22**, pp. 401–426.

- CHANCOGNE, M., GAUMONT, R. & GRISON, P. (1949). Différences de sensibilité de divers stades embryonnaires de la cheimatobie (*Operophtera brumata* L., lépidoptère géométride), à l'action du dinitrocrésylate de sodium.—C. R. Acad. Sci., Paris, **228**, pp. 776–777.
- DAVIES, L. (1948). Laboratory studies on the egg of the blowfly *Lucilia sericata* (Mg.).—J. exp. Biol., **25**, pp. 71–85.
- DIERICK, G. F. E. M. (1942). De ovicide werking van wintersproeimiddelen bestudeerd in het laboratorium.—117 pp. Assen, van Gorcum & Co.
- FINNEY, D. J. (1947). Probit analysis. London, Cambridge Univ. Press.
- * FOX, R. H. (1930). The action of certain oils on the egg-masses of the leaf roller *Archips argyrospila* Walk.—Thesis, Univ. New Hampshire.
- GAUMONT, R. (1951). Etudes embryologiques sur l'oeuf de cheimatobie, *Operophtera brumata* L., Lépidoptère Geometridae. Action de la température sur l'embryogenèse et action du dinitrocrésylate de sodium sur quelques stades embryonnaires.—Ann. Epiphyt., **1**, pp. 253–273.
- GIMINGHAM, C. T., MASSEE, A. M. & TATTERSFIELD, F. (1926). A quantitative examination of the toxicity of 3:5-dinitro-o-cresol and other compounds to insect eggs, under laboratory and field conditions.—Ann. appl. Biol., **13**, pp. 446–465.
- GIMINGHAM, C. T. & TATTERSFIELD, F. (1927). Laboratory and field experiments on the use of 3:5-dinitro-o-cresol and the sodium salt for winter spraying.—J. agric. Sci., **17**, pp. 162–180.
- GROSS, J. B. & HOWLAND, R. B. (1940). The early embryology of *Prodenia eridania*.—Ann. ent. Soc. Amer., **33**, pp. 56–76.
- HOUGH, W. S. (1939). Dinitro-o-cresol, dinitro-o-cyclo-hexylphenol and lauryl rhodanate in dormant sprays against eggs of apple aphids.—J. econ. Ent., **32**, pp. 264–270.
- JAHN, T. L. (1935a). The nature and permeability of grasshopper egg membranes. I. The EMF across membranes during early diapause.—J. cell. comp. Physiol., **7**, pp. 23–46.
- JAHN, T. L. (1935b). Nature and permeability of grasshopper egg membranes. II. Chemical composition of membranes.—Proc. Soc. exp. Biol. Med., **33**, pp. 159–163.
- JOHANNSEN, O. A. & BUTT, F. H. (1941). Embryology of insects and Myriapods. . .—462 pp. New York, McGraw-Hill.
- KORSCHULT, E. (1884). Ueber die Bildung des Chorions und der Micropylen bei den Insecteneiern.—Zool. Anz., **7**, pp. 394–398, 420–425.
- KORSCHULT, E. (1887). Zur Bildung der Eihüllen, der Mikropylen und Chorionanhänge bei den Insekten.—Nova Acta Leop. Carol., **51**, pp. 181–252.
- LEES, A. D. & BEAMENT, J. W. L. (1948). An egg-waxing organ in ticks.—Quart. J. micr. Sci., **89**, pp. 291–332.
- LEUCKART, R. (1855). Ueber die Micropyle und den feinern Bau der Schalenhaut bei den Insecteneiern.—Müller's Archiv Anat. Physiol., **1855**, pp. 90–264.
- LISON, L. (1936). Histo-chimie animale.—320 pp. Paris, Gauthier-Villars.
- LLOYD, Ll. (1920). The habits of the glasshouse tomato moth, *Hadena* (*Polia*) *oleracea*, and its control.—Ann. appl. Biol., **7**, pp. 66–102.
- LORD, K. A. (1949). The effect of insecticides on the respiration of *Oryzaephilus surinamensis*: an attempt to compare the speeds of action of a number of DDT analogues.—Ann. appl. Biol., **36**, pp. 113–138.
- LORD, K. A. (1950). The effect of insecticides on respiration. II. The effects of a number of insecticides on the oxygen uptake of adult *Tribolium castaneum* Hbst., at 25°C.—Ann. appl. Biol., **37**, pp. 105–122.

- LORD, K. A. & POTTER, C. (1951). Studies on the mechanism of insecticidal action of organo-phosphorus compounds with particular reference to their anti-esterase activity.—Ann. appl. Biol., **38**, pp. 495–507.
- LUDWIG, D. (1946). The effect of DDT on the metabolism of the Japanese Beetle, *Popillia japonica* Newman.—Ann. ent. Soc. Amer., **39**, pp. 496–509.
- MAERCKS, H. (1935). Ueber die Wirkung von Nikotin und Pyrethrum auf die Eier des Apfelwicklers (*Carpocapsa pomonella* L.) und des bekrenzten Traubenwicklers (*Polychrosis botrana* Schiff.).—Anz. Schädlingssk., **11**, pp. 13–19. (R.A.E., (A), **23**, p. 226.)
- MATTHÉE, J. J. (1951). The structure and physiology of the egg of *Locustana pardalina* (Walk.).—Sci. Bull. Dep. Agric. S. Afr., no. 316, 83 pp.
- METCALF, R. L. (1948). The mode of action of organic insecticides.—Rev. chem.-biol. Co-ord. Cent., no. 1, 84 pp. Washington, D.C.
- * MORI, H. (1940). Lethal effect of contact insecticides upon insect eggs. 1. Pyrethrin. [In Japanese.]—Oyo. Dobuts. Zasshi, **12**, pp. 209–214. (R.A.E., (A) **30**, p. 17.)
- MUKERJEA, T. D. (1953). The relationship between the stage of development and susceptibility to DDT and the pyrethrins of *Diatraxia oleracea* (L.), *Tenebrio molitor* L., and *Periplaneta americana* (L.).—Bull. ent. Res., **44**, pp. 121–161.
- MÜLLER, K. (1938). Histologische Untersuchungen über den Entwicklungsbeginn bei einem Kleinschmetterling (*Plodia interpunctella*).—Z. wiss. Zool., (A) **151**, pp. 192–242.
- MUSGRAVE, A. J. (1937). The histology of the male and female reproductive organs of *Ephestia kühniella* Zeller (Lepidoptera). I. The young imagines.—Proc. zool. Soc. Lond., (B) **107**, pp. 337–364.
- NORRIS, M. J. (1932). Contributions towards the study of insect fertility. I. The structure and operation of the reproductive organs of the genera *Ephestia* and *Plodia* (Lepidoptera, Phycitidae).—Proc. zool. Soc. Lond., **1932**, pp. 595–611.
- O'KANE, W. C. & BAKER, W. C. (1934). A technique for tracing penetration of petroleum oil in insect eggs. . . Studies of contact insecticides VIII. Part 1.—Tech. Bull. N.H. agric. Exp. Sta., no. 60, pp. 1–7.
- O'KANE, W. C. & BAKER, W. C. (1935). Further determinations of oil penetration into insect eggs. Studies of contact insecticides IX.—Tech. Bull. N.H. agric. Exp. Sta., no. 62, 8 pp.
- * ONOE, T. & FUKUDA, J. (1939). Effectiveness of pyrethrum against the eggs of *Chilo simplex* Butl. [In Japanese.]—Oyo Dobuts. Zasshi, **11**, pp. 146–147. (R.A.E., (A) **27**, p. 620.)
- PETTY, B. K. (1948). Laboratory experiments with new organic insecticides.—Fmg in S. Afr., **23**, pp. 325–332.
- POTTER, C. (1952). An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids.—Ann. appl. Biol., **39**, pp. 1–28.
- POTTER, C. & TATTERSFIELD, F. (1943). Ovicidal properties of certain insecticides of plant origin. (Nicotine, pyrethrins, derris products).—Bull. ent. Res., **34**, pp. 225–244.
- SLIFER, E. H. (1930). Insect development. I. Fatty acids in the grasshopper egg.—Physiol. Zool., **3**, pp. 503–518.
- SLIFER, E. H. (1937). The origin and fate of the membranes surrounding the grasshopper egg; together with some experiments on the sources of the hatching enzyme.—Quart. J. micr. Sci., **79**, pp. 493–506.

- SLIFER, E. H. (1938). The formation and structure of a special water-absorbing area in the membranes covering the grasshopper egg.—*Quart. J. micr. Sci.*, **80**, pp. 437–458.
- SLIFER, E. H. (1948). Isolation of wax-like material from the shell of the grasshopper egg.—*Disc. Faraday Soc.*, no. 3, pp. 182–187.
- SLIFER, E. H. (1949a). Changes in certain of the grasshopper egg coverings during development as indicated by fast green and other dyes.—*J. exp. Zool.*, **110**, pp. 183–203.
- SLIFER, E. H. (1949b). Variations, during development, in the resistance of the grasshopper egg to a toxic substance.—*Ann. ent. Soc. Amer.*, **42**, pp. 134–140.
- SPEYER, E. R. & PARR, W. J. (1945). Animal pests. 4. Tomato moth (*Polia oleracea* L.).—30th Rep. exp. Res. Sta. Cheshunt, 1944, pp. 38–39.
- TATTERSFIELD, F., GIMINGHAM, C. T. & MORRIS, H. M. (1925). Studies on contact insecticides. Part III. A quantitative examination of the insecticidal action of the chlor-, nitro- and hydroxyl derivatives of benzene and naphthalene.—*Ann. appl. Biol.*, **12**, pp. 218–262.
- VERSON, E. (1893). Dei canali aeriferi che attraversano nel filugello il guscio dell'ovo.—*Staz. sper. agr. ital.*, **24**, pp. 9–12.
- VIEL, G. & CHANCOGNE, M. (1951). Etude des actions ovicides. I. Techniques d'essai.—*Ann. Epiphyt.*, **1**, pp. 293–306.
- WAY, M. J., SMITH, P. M. & HOPKINS, B. (1951). The selection and rearing of leaf-eating insects for use as test subjects in the study of insecticides.—*Bull. ent. Res.*, **42**, pp. 331–354.
- WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects.—*J. exp. Biol.*, **21**, pp. 97–114.
- WIGGLESWORTH, V. B. & BEAMENT, J. W. L. (1950). The respiratory mechanisms of some insect eggs.—*Quart. J. micr. Sci.*, **91**, pp. 429–452.
- ZIMMERMAN, P. W. & HARTZELL, A. (1947). Hexaethyl tetraphosphate and tetraethyl pyrophosphate: I. Their effects on plants. II. Their toxicities to insects and mites.—*Contrib. Boyce Thompson Inst.*, **15**, pp. 11–19.
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Saissetia zanzibarensis sp. n. (fig. 1).

The mature adult female becomes highly convex in a similar manner to that of *S. coffeae* (Wlk.) (= *hemisphaerica* Targ.). The integument is slightly rugose and of a yellow-brown to a dark brown colour and there is a slight outward curve at the sides of the anal cleft giving the insect a characteristic appearance. Length 3 mm., width 2 mm.

Antennae with eight segments. Legs well developed, tibiotarsal articulation without articulatory process. Marginal setae numerous, each being slender, pointed and unusually long. There appears to be an inner and outer row of these marginal setae, those of the inner row being shorter and alternating, for the most part, with those of the outer row. Stigmatic setae in groups of three, each group comprising two short setae between which is a long, stout, pointed seta of the same length as those on the margin. Ventrally there is a submarginal row of minute setae.

Anal plates quadrate when together, each plate with outer margins roughly equal in length. A pair of slender apical setae to each plate, the dorsal surface also bearing a stout, pointed, discal seta and between this and the apical setae lie five or six smaller setae. Two groups of five setae are present on the postero-ventral margin of the anal invagination. Dorsal surface of integument with very small round pores, these in the mature female becoming surrounded by circular or oval areas which are always paler than the rest of the integument. About twenty tubercle-like pores situated anterior to the anal plates. Small pointed setae scattered over the surface in no definite pattern. Submarginal tubercles apparently absent. Ventral tubular ducts numerous in a submarginal zone extending around the body. The ducts all of one shape, each with a slender apical filament. Multilocular disc pores situated at the base of the anal cleft only. Quinquelocular stigmatic pores more or less in a double row.

The specimen selected as "type" is a young adult female from *Eugenia jambos*,³ collected by M. J. Way, Dole, Zanzibar, 25.V.51. Deposited in the British Museum (Nat. Hist.).

The specimen figured (from *Eugenia jambos*³) is one taken before maturity, while the integument is membranous.

This species is widely distributed in Zanzibar and Pemba and recently it has been collected on *Mangifera indica* in association with *Oecophylla longinoda* at Mvomero, 40 miles north of Morogoro in Tanganyika Territory. It is very common on the clove tree but is also found on a wide variety of hosts. Specimens have been examined from *Eugenia aromatica*,¹ *E. jambolana*,² *E. jambos*,³ *Mangifera indica*, *Canarium commune*, *Persea americana*, *Cassia* sp., *Gliricidia sepium*, *Psidium guajava*, *Averrhoa carambola*, *Cocos nucifera*, *Citrus* sp., *Achras zapota*,⁴ *Adansonia digitata* and *Ficus* sp. The insects occur mainly on the small shoots and on the clove tree they are in particular evidence on the young inflorescences.

NOTES. This species comes close to *Saissetia coffeae* (Wlk.) in general appearance but it differs in the number and shape of the marginal setae and in carrying numerous dorsal setae on the anal plates. The tubular ducts differ in that they are all of one shape.

Reference.

NUTMAN, F. J. & SHEFFIELD, F. M. L. (1949). Studies of the Clove Tree.—Ann. appl. Biol., **36**, pp. 419-439.

^{1, 2, 3, 4} On the authority of the Director of the Royal Botanic Gardens, Kew, it is understood that these are now known as *Jambosa caryophyllus*, *Syzygium cumini*, *Jambosa jambos*, and *Achras zapotilla*, respectively.

2 STUDIES OF BRITISH ANTHOMYIID FLIES.

By Mary MILES.

Wye College (University of London).

V. THE ONION FLY, *Delia antiqua* (Mg.).

E.M.N.

The onion fly, *Delia antiqua* (Mg.), is a well-known pest of onions, leeks and shallots in Europe and North America. It is widely distributed in Britain but its occurrence is erratic, and in localities known to the writer (for example, Fladbury, Wores. and Wyē, Kent) where onions and leeks have had a regular place in the crop rotation on extensive market gardens for many years, a careful search has failed to reveal its presence. Other Anthomyiid larvae, in particular those of the bean seed flies, *D. cilicrura* (Rond.) and *D. trichodactyla* (Rond.), also attack onions and leeks and cause serious injury to seedlings and newly transplanted crops. Differences in the pattern of attack by the several species make it necessary to distinguish their immature stages.

Eggs.

The eggs (fig. 1) are white, elongate, with one side convex and the other slightly concave. The chorion is finely reticulated but the longitudinal striation is more marked and gives the appearance of broken ribbing. The anterior end is truncate with a rim that partly encloses the micropylar area and extends along the concave surface as a pair of diminishing ridges for about a quarter of the length. A series of 8 eggs laid by a captive female had an average length of 1.2 mm. This agrees with the length 1.12–1.23 mm. given by Maan (1945).



Fig. 1.—Eggs of onion fly ($\times 20$).

Comparison of eggs of *D. antiqua* with those of *D. cilicrura* shows that they are similar in appearance but those of *D. antiqua* are larger. Miles (1952) found that eggs of *D. cilicrura* had an average length of 0.92 mm. and that none of the eggs measured were more than 0.95 mm. long. Neither Maan (*op. cit.*) nor the writer has found eggs of *D. antiqua* less than 1.12 mm. long. This difference in size should be a fairly reliable distinguishing character because adults of *D.*

antiqua are normally 6–7 mm. long while adults of *D. cilicrura* are usually only 4–5 mm. long.

The positions, numbers and distribution of the eggs are also a valuable aid to their identity. Eggs of *D. antiqua* are laid on or near the host plants and, if attack by this species is imminent, considerable numbers of eggs will be found about the crop. On the other hand, eggs of *D. cilicrura* and *D. trichodactyla* are usually widely distributed in the soil where they are rarely seen, and though they are sometimes laid in small numbers in the same positions as those of *D. antiqua* this is not usual.

Larvae

Larvae of *D. antiqua* are whitish legless maggots with the head retracted into the prothorax, and the tip of the body triangular in profile with an apical ridge bearing four distinct sub-conical tubercles. The anus is on the ventral surface on the anal area which appears to be the rudimentary 9th abdominal segment. The three instars are distinguishable by the numbers of openings in the posterior spiracles and by the character of the bucco-pharyngeal armature. Mature larvae of *D. antiqua* are 9 mm. long.

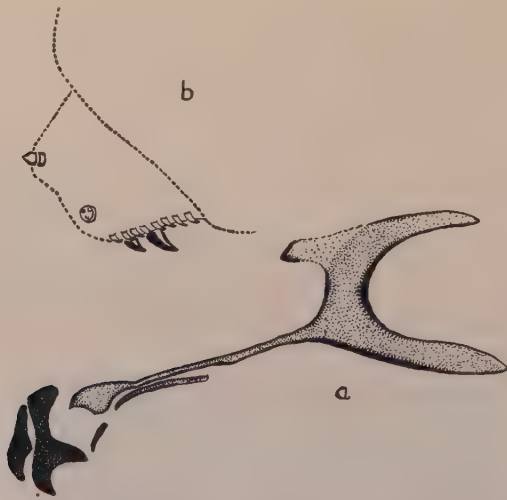


Fig. 2.—a. Cephalo-pharyngeal skeleton of first instar larva of *D. antiqua* ($\times 180$). b. Diagram of exerted head to show oral spines.

In the first instar each mandible consists of two separate crotchets and a delicate H-shaped hypostomal sclerite fused with the pharyngeal sclerite (fig. 2). In the integument on each side of the mandibles there are 8–10 oral spines (fig. 2b). Miles (1952) has illustrated the bucco-pharyngeal armature of the first-instar larva of *D. cilicrura*; it appears indistinguishable from that of *D. antiqua*.

In the second instar the mandible consists of a single crocket. In fig. 3 it is shown with two ventral teeth because most of the larvae examined had this character. Other larvae had three ventral teeth and the vestiges of a fourth tooth were sometimes visible. Maan (*op. cit.*) shows the mandible with four ventral teeth. It seems therefore that the form of the mandible in the second instar is variable and there may be 2–4 teeth on the ventral surface. The accessory sclerite associated with the ventral process varies in size and is often indistinct owing to dense sclerotisation of the surrounding area.

A comparison of the bucco-pharyngeal armature of the second-instar larva of *D. antiqua* with that of second-instar larvae of *D. cilicrura* and *D. trichodactyla* shows that they are similar. Brooks (1951) illustrated the second-instar larva of *D. cilicrura* with three ventral teeth and Miles (1952) showed four, and examination of more larvae has shown that the number varies but no

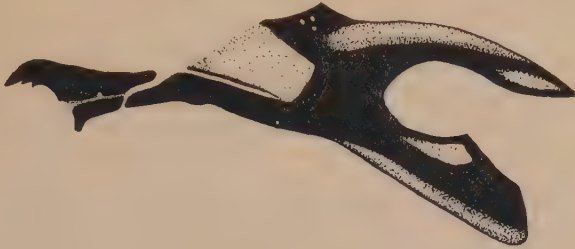


Fig. 3.—Cephalo-pharyngeal skeleton, second instar larva of *D. antiqua* ($\times 100$).

specimens with only two ventral teeth have been observed. *D. cilicrura* has two accessory sclerites but as Brooks (*op. cit.*) showed, the area is subject to heavy sclerotisation which may make the dentate sclerites indistinct as in *D. antiqua*.

In the third-instar larva of *D. antiqua* (fig. 4) the mandible is a stout crotchet with one accessory sclerite. It is similar in form to that of *D. cilicrura* but the writer has not yet observed whether, like that of *D. cilicrura*, the developing mandible of *D. antiqua* has the ventral surface serrated.

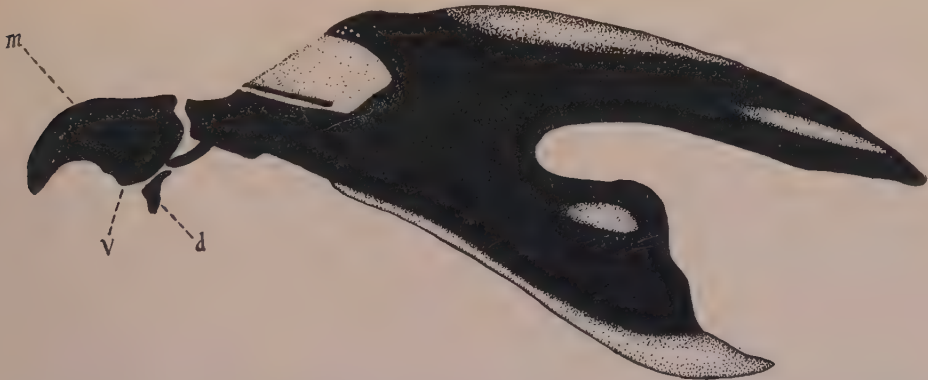


Fig. 4.—Cephalo-pharyngeal skeleton. Third instar larva *D. antiqua* ($\times 125$). (m, mandible; v, ventral process of the mandible; d, accessory sclerite).

The eighth abdominal segment bears eight pairs of subconical tubercles, the arrangement of which is shown in fig. 5. The tubercle pattern of *D. antiqua* is similar to that of *D. cilicrura* (Miles, 1952). The two pairs of apical tubercles are large and prominent and the ventro-apical tubercles are small and distinct.

In the second- and third-larval instars there are anterior spiracles at the junction of the pro- and meso-thoracic segments. In *D. antiqua* they have 10–13 finger-like processes, 11 or 12 processes occurring most frequently. In larvae of *D. cilicrura* and *D. trichodactyla* the anterior spiracles have 5–8 processes, 6 or 7 processes occurring most frequently. This difference in the number of

processes of the anterior spiracles is the only character so far observed by which the writer is able to distinguish larvae of *D. antiqua* from those of *D. cilicrura* and *D. trichodactyla*.

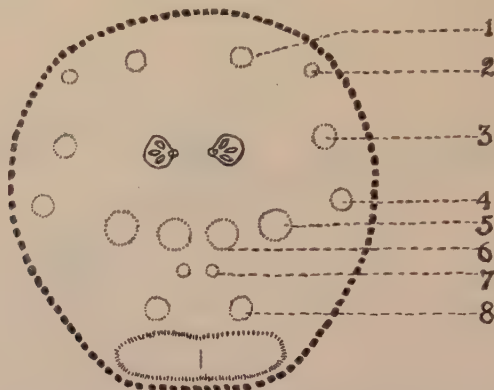


Fig. 5.—Posterior view (diagrammatic) of tip of abdomen of larva of *D. antiqua*. 1. dorso-central tubercles; 2. dorsal tubercles; 3. dorso-lateral tubercles; 4. lateral tubercles; 5. outer apical tubercles; 6. inner apical tubercles; 7. ventro-apical tubercles; 8. supra-anal tubercles.

Notes on the Habits.

It has not been possible to make field observations on *D. antiqua* because, as already stated, it has not so far been found in the vicinity of Wye College. Material for study was obtained from two localities in the west of England in late June and early July, and larvae from infested onions were allowed to complete their development in captivity.

Adults emerged in July and early August but approximately 20 per cent. of the puparia entered a diapause that continued through the autumn and winter. Maan also found that many of the larvae that attained maturity in the summer did not emerge as adults until the following year.

Newly emerged flies were kept in breeding cages 7 ins. square and 15 ins. high with wooden base, a top of metal gauze framed with wood, fixed glass panels on three sides, and in front a 2-inch strip of metal gauze at the base and a glass panel that slid upwards to permit the handling of the insects and their food and host plants. These cages were easy to manipulate, afforded suitable conditions for the insects and had sufficient space for a small pot containing a plant. Food for the flies was put on the floor of the cages in watch glasses with cotton wool. Onion flies in captivity fed readily on sugar solution, water containing fishmeal (poultry food) and on cow dung kept moist by a film of water. Eggs were laid by flies with access to sugar solution and water containing fish meal. Kästner (1929) and, later, Maan (*op. cit.*) also found that a diet of sugar and casein was necessary to stimulate egg-laying in captive flies. At Wye egg-laying took place in the laboratory; it was not necessary to put the cages out of doors in the morning sunshine as Maan suggested.

Pattern of Attack on Field Crops.

The onion flies and bean seed flies have different habits and consequently the circumstances of their attacks on susceptible crops differ greatly.

Onion flies are attracted to *Allium* spp. for egg-laying and subsequently the larvae feed on these plants. Records of larvae of onion flies from other host plants exist but they are not numerous and their reliability is doubtful. It is reasonable, therefore, to consider the onion fly to be a specific pest whose

presence in a crop is subsequent to the germination of the seeds or the planting of small sets.

The larvae of the two bean seed flies, which are similar in appearance and habit, are primarily saprophytic in the soil but they feed so generally that they have been recorded from almost all extensively cultivated plants and from buried crop refuse. They also occur as predators on other insects and they are capable of reaching maturity on organic fertilisers. The flies are attracted to freshly worked soil and when there is no crop they lay eggs in the open among the soil particles. When crops are present the eggs are often deposited in the vicinity of the plants, probably because they afford shade and shelter for the flies. Attack on onions and leeks by bean seed flies may occur as the result of infestation of the soil prior to sowing or planting or as a result of cultural operations afterwards. The former is more likely to be serious because the larvae may destroy the seedlings before they break the soil.

Attack by onion flies and bean seed flies may occur at any time of the year from April to October. Attack by onion flies is most likely to occur in spring and early summer because their numbers appear to be greatest and conditions are usually most favourable for their attack in the first half of the season. Infestation by bean seed flies is less likely in spring because the preparation of seed beds usually takes place in March before the overwintering flies emerge, and the present practice is to destroy weeds by chemical means instead of by cultivation and this avoids the disturbance of the soil and the consequent assembling of the flies at the young crop.

Damage to summer-sown onions is frequently observed in August and early September. The injury is usually ascribed to the onion fly, *D. antiqua*, but is much more likely to be the result of attack by the larvae of bean seed flies. Dr. H. C. Gough (personal communication) found that bean seed flies caused damage to seedling onions in the Eastern Province of the National Agricultural Advisory Service in August 1951, and it is probable that this injury is more widespread and common than has been recognised. The cultivations necessary for the preparation of a suitable seed bed for onions are often carried out in bright warm weather in late July and early August when the third generation of bean seed flies is emerging, and the flies are readily attracted from the shelter of nearby crops and headlands to the moist, freshly turned soil. The poor germination of onion seed, sometimes recorded at this time of the year may be the result of the feeding of the larvae of bean seed flies on the germinating seeds. Onion flies, on the other hand, are unlikely to be attracted from the mature crops on which they are already established and which usually remain in the fields throughout August.

Summary.

Onions and related crops are attacked by the onion fly, *D. antiqua* (Mg.), and by the bean seed flies, *D. cilicrura* (Rond.), and *D. trichodactyla* (Rond.). The eggs, larvae and puparia of the three species are similar in form, and the only morphological difference so far observed is in the number of processes of the anterior spiracles of the larvae. In *D. antiqua* the anterior spiracles have 10-13 processes; in *D. cilicrura* and *D. trichodactyla*, the immature stages of which cannot yet be distinguished from each other, the anterior spiracles have 5-8 processes.

Onion flies and bean seed flies have different habits and consequently the circumstances of their attacks differ. The onion fly is a specific feeder and its attacks are likely to be serious in spring and early summer. The bean seed flies are general feeders and attack on onions in spring is unlikely because the preparation of the soil, which attracts the flies, is usually completed before they emerge from the over-wintering puparia. Bean seed fly attack on onions

is likely to take place in August when onions for bunching are sown. The preparation of the seed bed attracts the flies of the third generation from the surrounding crops and the soil may become heavily infested with larvae which subsequently destroy the germinating seeds. In August and September it is unlikely that young crops will attract onion flies from the mature crops on which they are established.

Acknowledgements.

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References.

- BROOKS, A. R. (1951). Identification of the root maggots (Diptera: Anthomyiidae) attacking cruciferous garden crops in Canada, with notes on biology and control.—*Canad. Ent.*, **83**, pp. 109–120.
- KÄSTNER, A. (1929). Untersuchungen zur Lebensweise und Bekämpfung der Zwiebelfliege (*Hylemyia antiqua* Meigen). II. Teil. Morphologie und Biologie.—*Morph. Ökol. Tiere*, **15**, pp. 363–422.
- MAAN, W. J. (1945). Biologie en phaenologie van de uienvlieg, *Chortophila antiqua* (Meigen) en de preimot, *Acrolepia assectella* (Zeller), als grondslag voor de bestrijding.—*Meded. TuinbVoorlichtingsdienst*, no. 39, 92 pp.
- MILES, M. (1952). Studies of British Anthomyiid flies. III. Immature stages of *Delia cilicrura* (Rond.), *D. trichodactyla* (Rond.), *Erioischia brassicae* (Beh.), *E. floralis* (Fall.) and *Pegohylemyia fugax* (Mg.).—*Bull. ent. Res.*, **43**, pp. 83–90.
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AIRCRAFT APPLICATIONS OF INSECTICIDES IN EAST AFRICA.

I.—PRELIMINARY EXPERIMENTS IN AREAS SUPPORTING POPULATIONS OF THE TSETSE FLY (*GLOSSINA PALPALIS* (R.-D.)).

By K. S. HOCKING and D. YEO.

Colonial Insecticide Research Unit, Arusha, Tanganyika.

In 1945 the Colonial Insecticide Research Unit was set up by the Colonial Office to study the use of insecticides against insect pests in tropical Africa. Since 1948 a considerable amount of work has been done to investigate the possibilities of applying insecticides from aircraft. This work has been concerned mainly with tsetse flies, although some experiments have been done against mosquitos and other pests.

This paper describes aircraft applications of insecticides in areas supporting populations of the tsetse fly, *Glossina palpalis* (R.-D.). The experiments were designed to explore in a preliminary fashion the technical aspects of the method, and to compare certain formulations and drop distributions, rather than to attempt to eradicate tsetse flies. Four small islands (fig. 1) were treated. Nfo and Sowe were treated with coarse aerosols of BHC and DDT, respectively, and Tavu and Kimi with coarse sprays of oil-in-water emulsions of BHC and DDT respectively. It was thus possible to compare the effectiveness of the two insecticides applied as coarse sprays and as coarse aerosols.

There are two main features in the life cycle of a tsetse fly that determine the frequency with which insecticidal applications must be made, and the period that they must cover: the interval between emergence of a female fly and her first larviposition, and the pupal period. If adult flies are killed by an immediate contact effect, applications must be repeated at intervals less than this period between emergence and larviposition, so that females that emerge after an application are killed before they can larviposit. If flies are killed by means of residual deposits upon vegetation, the period between applications during which the deposits are ineffective must also be less than this interval between emergence and larviposition. Even if applications were to produce a complete mortality of all adult flies it would be necessary to continue for a complete pupal period to ensure that adults had emerged from all pupae laid down before the applications started. Since in field work an application of insecticide does not usually give a complete kill, it is desirable to cover at least two pupal periods to obtain a sufficiently large reduction in a population of tsetse flies.

The aerosols were used to produce an immediate contact kill, whereas the emulsions were applied to give residual lethal deposits upon vegetation. With the dosages and insecticides used in these experiments, residual deposits would not remain active for more than a few days, and it was decided to use the same interval between applications for the coarse sprays as for the aerosols. From temperature records taken in the area it was estimated that the pupal period would be approximately seven weeks, and that a female fly would produce her first larva 2-3 weeks after emergence. Each island was therefore treated with eight applications, at intervals of two weeks.

Description of the Area.

The islands were near the northern shore of Lake Victoria, and were only a few miles from the excellent aerodrome at Entebbe (Lat.-00°02'N, Long.-

32°26'E.). A map of the area, showing the positions of the islands, is given in fig. 1. From some points of view they were ideal, since they were small, isolated from other tsetse-infested areas, and supported dense populations of *G. palpalis*. On the other hand, they were specialised areas, representative only of the



Fig. 1.—A map of the area, showing the islands which were treated, the areas used for control catches of flies, and the aerodrome at Entebbe.

habitats of this particular species of tsetse fly, and were very different from areas in which live the economically more important species *G. morsitans* Westw., *G. swynnertoni* Aust. and *G. pallidipes* Aust.

The islands varied in size from about 100 to 250 acres. The vegetation on a considerable proportion of the area of each island consisted of reeds. There was a swampy area, containing papyrus (*Cyperus papyrus*) and ambatch (*Herminiera elaphroxylon*), and in some places *Phragmites* grass and *Afromomum* sp. The firm ground of the islands was bordered by a belt of low trees and shrubs, with many *Alchornea cordata* and *Triumfetta macrophylla*; inside this belt was dense high-canopied rain forest. Apart from Tavu, each island had a small grassy clearing of a few acres.

Insecticidal Formulations.

Unpublished work had shown that oil-in-water emulsions were better at producing residual deposits than either oil solutions, which are absorbed by the

leaves, or water suspensions, which are washed off by rain. They were prepared from concentrates supplied by the Shell Petroleum Company. The DDT concentrate (T.P.685) contained 20 per cent. w/v of technical DDT, and the BHC concentrate (T.P.694) contained 20 per cent. w/v of crude BHC. Both concentrates were made up with water to give a 2.5 per cent. w/v concentration of the crude insecticide; the final emulsions then contained either 0.20 lb. per gallon of the p,p' isomer of DDT or 0.032 lb. per gallon of the γ isomer of BHC.

The coarse aerosols consisted of oil solutions, and the solvent was usually 1 part Shell Furnace Oil and 4 parts Shell Power Kerosene although, for the last two applications upon Sowe, Shell M.K.E. was used instead of the Power Kerosene. On Nfo, a 5 per cent. w/v solution of the I.C.I. concentrate S.G. 215 (26 per cent. γ isomer BHC) was used. For the first six applications upon Sowe the solution was a 10 per cent. w/v solution of technical DDT; the solution strength was doubled in the last two applications.

The emulsions were easily prepared, but care was necessary to ensure that they were only left standing in the aircraft storage tanks for a few hours; appreciable sedimentation of the DDT occurred if they stood for more than 24 hours. During preparation of the oil solutions for the aerosols, the solvents were heated to ensure that all the insecticide went into solution.

The Control of Dosage.

Other work had suggested that the γ isomer of BHC was some six times as effective in killing tsetse flies as the p,p' isomer of DDT. Originally, therefore, it was intended to treat the islands with 1 gallon per acre of the emulsions or 0.25 gallons per acre of the oil solutions, which would give dosages of either 0.2 lb. per acre of the p,p' isomer of DDT or 0.032 lb. per acre of the γ isomer of BHC. In fact, however, dosages were increased for the later applications, and were generally low during the earlier applications; details of the nominal dosages are given in Table I. The nominal dosage is the theoretical dosage applied to an area as calculated from the speed of the aircraft and the rate of emission of insecticide. It differs from the actual dosage received on any area, which depends upon the meteorological conditions and the screening effect of vegetation.

TABLE I.

The nominal dosages used for the various applications upon the islands. The figures are given as gallons of solution per acre or lb. of insecticide per acre.

Application	Coarse aerosols				Emulsions			
	Nfo		Sowe		Kimi		Tavu	
	Vol.	γ isomer BHC	Vol.	p,p' isomer DDT	Vol.	p,p' isomer DDT	Vol.	γ isomer BHC
1	0.19	0.024	0.21	0.17	0.98	0.20	1.20	0.037
2	0.20	0.025	0.25	0.20	1.00	0.20	1.50	0.046
3	0.20	0.025	0.21	0.17	1.08	0.22	1.20	0.037
4	0.22	0.027	0.19	0.15	0.82	0.16	1.14	0.035
5	0.16	0.020	0.20	0.16	1.02	0.20	0.84	0.026
6	(0.32)	(0.040)	0.40	0.32	0.63	0.13	1.01	0.031
7	0.33	0.041	0.35	0.56	1.66	0.33	1.95	0.061
8	0.33	0.041	0.39	0.62	(1.02)	(0.20)	1.70	0.053

These nominal dosages differed from the intended dosages given in the preceding paragraph.

Aircraft apparatus.

Two Anson Mark I aircraft were used in the experiments. Each was fitted with two banks of storage tanks, consisting of a lower tank capable of containing 61 gallons, and an upper tank which could hold 27 gallons when the aircraft

was on the ground. Each aircraft was therefore capable of carrying nearly 180 gallons of insecticidal solution, but considering the height of the area above sea level (3,750 ft. approximately) and its effect upon the performance of the aircraft, only rarely were more than 150 gallons carried.

The emulsions were emitted, under gravity, through two wide pipes which extended about 14 inches below the fuselage of the aircraft. The orifices faced the tail of the aircraft, and the drop distribution was produced by the interaction of the slipstream and the column of liquid. An iris diaphragm was fitted to the end of each emission pipe, by which the orifice area, and consequently the emission rate, could be varied; it was fitted with Bowden cable controls so that the pilot was able to vary the emission rate during a flight. A complete description of the installation is given on page 96 and in fig. 40 of a paper by Gunn and others (1948a).

The coarse aerosol was produced by allowing the solution to flow into modified exhaust systems of the aircraft engines. The solution flowed through a narrow pipe, 7/8 in. in internal diameter, into the exhaust pipe at a distance of $19\frac{1}{2}$ in. from the centre line of the exhaust manifold. It then passed down long exhaust tubes, 4 in. in diameter, and was finally emitted vertically downwards into the slipstream about 1 ft. below the trailing edge of the wing. The installation was based upon a design used for similar work against tsetse flies in S. Africa (du Toit & Kluge, 1947).

Both the spray and the aerosol systems were gravity-fed, and the emission rates fell as the storage tanks emptied. The variation was slight for the spray installation if each bank of tanks contained more than 20 gallons; for smaller amounts than this the emission rate fell rapidly until, when the tanks were nearly empty, the emission rate was only 30–40 per cent. of its original value. The aircraft had several gallons left after an application in most cases, and it was not considered worthwhile to reset the iris diaphragm during flight. The emission rate of the aerosol installation varied by approximately 10 times as the tanks emptied, and there was no way in which this variation could be eliminated. However, usually less than 30 gallons were used from each bank of tanks; by filling the tanks with 60 gallons for each bank, variations were reduced to ± 30 per cent. of the average value. In view of the exploratory nature of the experiments this variation was accepted. In later papers, however, it will be shown that the wide variation in emission rate with the aerosol apparatus complicated the treatment of large areas of savannah woodland, when the full aircraft payload had to be used.

Average nominal dosages did in fact vary considerably from the dosages which had been intended, as may be seen from Table I. This was mainly due to difficulties in setting the iris diaphragm accurately, or to partial blocking of the feedpipe to the exhaust pipes. The apparent variation was also increased by errors in reading the relatively inaccurate dipsticks which were used to measure the amount of solution in the storage tanks.

Field methods.

The coarse sprays were applied according to principles developed at Porton, England; these were used and fully described by Gunn and others (1948a, 1948b). A drop spectrum determination showed that a height-wind product of 2,000 (ft. \times m.p.h.) was desirable, with a swathe of 60 yards. Winds during the early morning were light and would have given aircraft heights of over 400 ft. on many occasions. Instead, the applications were carried out during the late afternoon, when winds were such that aircraft heights were usually 200–300 ft.

To apply the aerosols the aircraft was flown as close as possible to the top of the tree canopy, and at right angles to the mean wind direction. The applications were made as soon as possible after dawn, so that the effect of atmospheric turbulence upon the aerosol behaviour would be as small as possible. A swathe

width of 55 yds. was used, but this was dictated mainly by the emission rate of the installation and was not chosen to give particular limits to the variations in dosage.

Marker buoys were placed along the side of the islands. A smoke generator, carried in a dinghy, indicated the start of an emission run; the aircraft was flown over this marker, on a compass course calculated from the required track, the mean wind speed and direction, and the aircraft airspeed. The runs were so short that no serious errors in tracking occurred even at the ends of the runs farthest from the marker.

When it became evident that the reductions in fly populations were negligibly small upon Tavu and Kimi, the two islands treated with the emulsions, it was decided to increase the dosage and change the method of application of the spray. This was done by doubling the emission rate on each run, keeping the swathe width constant; furthermore the Porton method was abandoned, and instead the aircraft was flown as close as possible to the top of the canopy. This gave swathes of heavy dosage, about 20–30 yds. wide, separated by areas which were only lightly dosed. In this way it was hoped to produce bands of heavier and more lethal deposits, and to reduce losses of insecticide; within such bands the average nominal dosage was 1.5–2.0 times that over the entire island. Due to an error the average nominal dosage was not increased for the eighth application upon Kimi.

Physical and Chemical Assessments.

Three main methods were used to assess the ground deposits of the sprays; direct estimation of the insecticide, colorimetric assessment of a fast dye added to the solution, and what may be called a stain size method. The first method is self-explanatory. It is, however, laborious and unsuitable for the estimation of large numbers of samples when only a limited staff is available. For the colorimetric method a fast water-soluble dye (Crocein Scarlet) was added to the solution, and samples taken from the treated area were compared, using a Spekker absorptiometer, with standard solutions made up from the solution in the aircraft tanks. The stain size method relies upon the fact that for oil-in-water emulsions, —and for certain oil solutions—coloured drops of the solution produce upon absorbent paper stains which are stable in size, and whose diameters are approximately a power function of the drop diameters. A graph was prepared relating stain and drop diameters for the emulsions; using this graph, and measuring the density of numbers of drops, deposits could then be estimated.

Most of the assessments of sprays were in fact carried out with the stain size method. This actually records the *volume* of the deposit, but there were no significant differences between results obtained by all three methods. For some island assessments no dye was available; and it was necessary to use the faint colours of the emulsions to determine stain sizes. This led to some increase in the inaccuracies of assessment.

The aerosols were assessed by measuring deposits upon glass plates coated with magnesium oxide; some assessments were also made with cascade impactors (May, 1945). The assessment techniques used during these experiments were in general inadequate, and it was not until later that ordered knowledge was obtained of aerosol behaviour.

No significant differences were found between the physical characteristics of solutions containing DDT or BHC, but it will be convenient to consider the sprays and the aerosols separately.

Coarse sprays.

Drop spectra determinations and ground recoveries.

Several ground recoveries and drop spectra determinations were made in open areas, and the relevant data are summarised in Table II.

TABLE II.
Principal constants of the drop spectra, and ground recoveries, of the coarse sprays.

Date	Air temp. at 6 ft. (in ° F.)	R.H. %	Wind Speed at 6 ft. (m.p.h.)	Aircraft Airspeed (m.p.h.)	Emission rate (gal./sec.)	Spraying Height (in ft.)	Formulation	Density at 62° F. (gm./cc.)	Viscosity at 80° F. (Redwood) (Seconds)	Millimetres			% Recovery
										25% Vol. Dia.	50% Vol. Dia.	75% Vol. Dia.	
August 1948	65	58	6	120	1.5	170	T.P. 685 (21% DDT)	1.03	27.5	0.47	0.70	1.02	82
August 1948	70	51	10	120	1.4	200	T.P. 694 (21% BHC)	1.00	26.5	0.49	0.70	0.98	67
1.11.48	79	56	7	(126)	1.1	130	T.P. 685 (21% DDT)	1.03	27.5	0.48	0.69	0.95	60
25.2.49	80	64	(1.7)	(126)	1.05	130	T.P. 694 (21% BHC)	1.00	26.5	0.48	0.74	1.02	63
25.2.49	82	61	3.7	(126)	1.05	210	T.P. 694 (21% BHC)	1.00	26.5	0.43	0.64	0.94	50
25.2.49	80	66	7	(126)	1.10	330	T.P. 694 (21% BHC)	1.00	26.5	0.53	0.78	1.10	41

The Table includes relevant field data.

A typical drop spectrum is shown in fig. 2, using log-probit paper. The assessments were made with the stain size method, and show a decrease in the recovery of liquid volume with an increase in spraying height (Table II). On selected rows of some of the experiments colorimetric and chemical assessments were made. These gave results which were not significantly different from the results obtained with the stain size method, although it is perhaps asking too much to expect to detect small differences in such work. The results do, however, suggest that there is a real reduction in recovery of insecticide as the aircraft height is increased, probably due to greater losses of droplets downwind from the experimental area with the greater spraying heights.

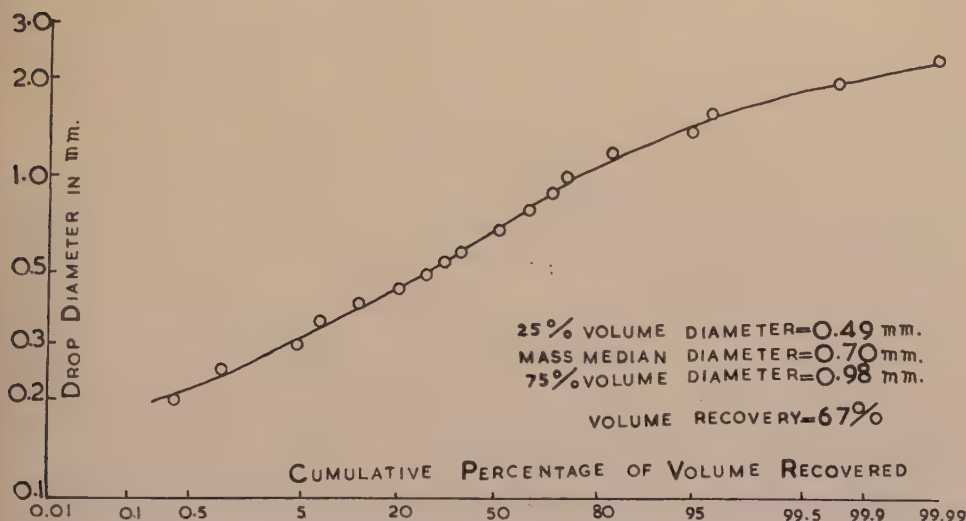


Fig. 2.—A typical drop spectrum of the ground deposit obtained in open areas from the coarse sprays. The assessment was made using the stain size method.

Island assessments.

Of the two islands treated with sprays, only Kimi had an open area in which ground recoveries could be measured. The results were not very reliable, since much of the area was shielded, particularly for the smaller drops, by the surrounding high canopy, but recoveries of liquid volume in the open varied from 28 to 46 per cent. with an average value of 41 per cent. The mean aircraft height was 250 ft., for which the complete recovery trials predict a recovery of approximately 45 per cent.

It was very difficult to estimate the vegetation cover in terms applicable to the behaviour of sprays. The canopy was dense on both islands, but it varied so much in type and density that only a general picture was obtained of the penetration by the sprays.

Tunnel paths were cut into the forest, and the ground deposit was measured at regular intervals of distance. Dosages varied enormously. In some sites they were comparable to the dosages in the open areas, whereas in other sites they were too small to be measured. In general, they fell into two groups; one group, representing approximately 20 per cent. of the forest area, were very little different from the dosages that were obtained in open sites, whereas the other group had dosages that varied from those too small to be measured to about 10 per cent. of dosages in open areas, with an average dosage of 2.5 per cent. of the nominal dosage.

An independent survey of the forest showed that about 20 per cent. of the area consisted of gaps in the canopy; it was also possible to correlate the high dosage with such gaps. The rest of the canopy was dense; a visual estimate of the cover, with a ruled grid, gave values varying from 80 to 100 per cent.

Thus, in open areas and for the outer layer of vegetation, dosages were 41 per cent. of the nominal dosages given in Table I. Within the canopy, over 80 per cent. of the area received dosages which were less than 5 per cent. of the nominal dosage, and the remaining sites within the canopy received dosages similar to those in open areas and coincided with gaps in the canopy. A spectrum with such large drops is therefore quite ineffective in penetrating a heavy forest canopy to produce a deposit upon the lower levels of vegetation. Since *G. palpalis* probably lives within a few feet of the ground, only some 20 per cent. of the fly haunts on Tavu and Kimi received doses of 41 per cent. of the nominal dosage; the remaining fly haunts received dosages which averaged only 2.5 per cent. of the nominal dosages.

Coarse aerosols.

It is difficult to measure the droplet distribution of an aerosol that contains droplets with diameters varying from a few to 100–200 microns. The larger droplets deposit quite readily, and can be measured upon oxide-coated plates placed upon the ground, but the small droplets do not deposit upon the ground

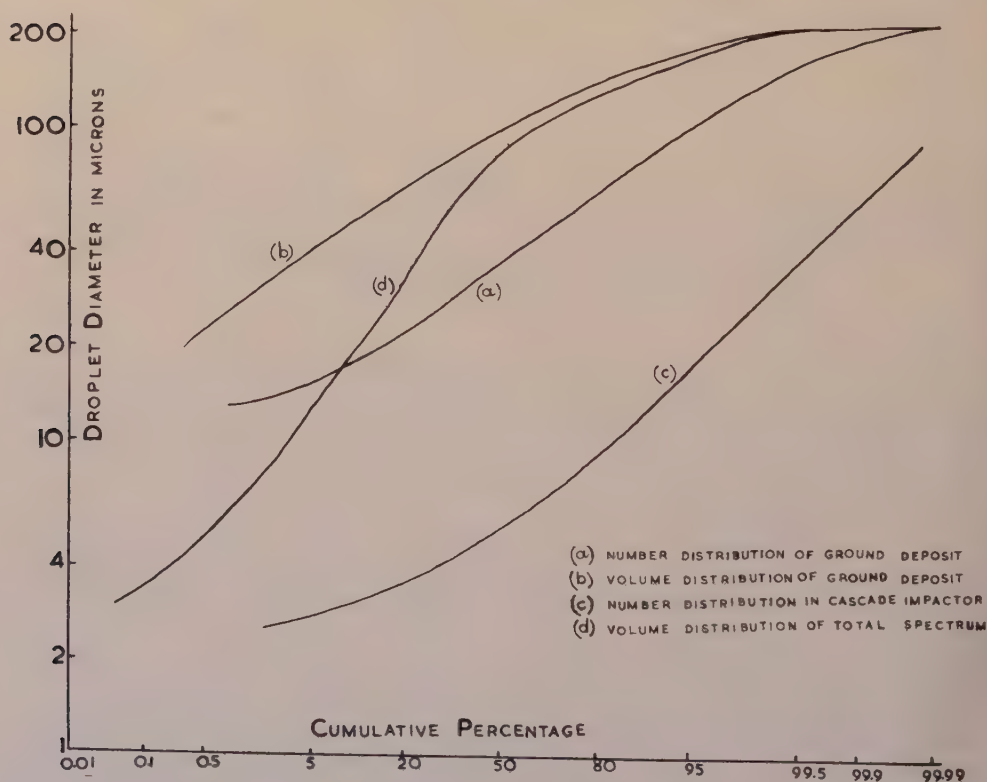


Fig. 3.—Typical drop spectra of the coarse aerosols. Graphs (a) and (b) are mean spectra of the ground deposit, measured upon oxide-coated plates; graph (c) is the number distribution of the sample collected by cascade impactors, and (d) is a crude graph of the volume distribution of the total spectrum.

in appreciable numbers within any measurable distance from the line of emission, and their distribution can only be measured with an instrument such as the cascade impactor, which does not sample accurately droplets greater than 50 microns in diameter.

The spectrum of the ground deposit, as measured upon the oxide-coated plates, is shown by (a) and (b) of fig. 3. The number distribution of the samples made by cascade impactors is shown by (c) of fig. 3 and is very different from that of the ground deposit. To obtain the complete spectrum it is necessary to equate the cascade impactor and ground deposit distributions in some way, and so far no satisfactory method has been found for doing this. The graph (d) of fig. 3 has been constructed by equating the two distributions over the range of diameters 40–50 microns, and gives some crude idea of the distribution of volume over the entire droplet range.

The oxide-coated plates and the cascade impactors record the volume of the samples. Colorimetric assessments showed that the solution of the ground deposit was approximately three times as concentrated as the solution in the aircraft storage tanks, so that some 70 per cent. of the solvent was lost by evaporation before the droplets deposited. They also showed that of the insecticide collected only 5 per cent. was in droplets less than 13 microns in diameter.

The ground recovery and its drop spectrum depend complexly upon field conditions, notably the degree of atmospheric turbulence and the type and density of vegetation cover (Yeo & Thompson, *in press*). For assessments made in open areas during the period soon after dawn the recoveries of liquid volume varied from 4 to 15 per cent. with an average of approximately 10 per cent., giving a probable ground recovery of insecticide of 30 per cent., for an aircraft height of 50 ft.

Only oxide-coated plates were used for assessing the aerosol behaviour during the applications on the islands. As with the sprays, dosages varied enormously. Some sample plates had no droplets upon them, even in open sites, although most droplet densities varied from $1\text{--}20 \times 10^4$ droplets per sq. yd. Under the canopy the droplets were more diffusely distributed than for the sprays, and averaged 30 per cent. of the numbers in open areas. Later work showed, however, that it was the smaller droplets that penetrated the canopy, and it is extremely improbable that ground recoveries in the forest area were more than 20 per cent. of recoveries in open sites. Since on only four out of 20 occasions when aerosol applications were attempted were conditions of high inversion and low wind speed obtained,—the atmospheric conditions necessary for large ground recoveries of insecticide,—it is improbable that ground recoveries of insecticides inside the canopy averaged more than 5–10 per cent. of the nominal area dosage.

Entomological Data.

Usual methods were employed to assess the tsetse populations. The islands were effectively covered with catching stations; at each station two fly catchers caught all flies for a period of 15 minutes. The catches were repeated daily. In Table III the weekly mean catch of non-teneral males per station is given. In addition the populations of male flies were estimated just before and soon after the series of applications, using a modified form of the method described by Jackson (1944). In this method flies caught in a particular week are marked with non-toxic oil paint of a fixed colour; the proportions of marked flies in the catches in subsequent weeks are recorded and from them it is possible to estimate the population during the weeks of marking. In our population estimates three colours were used; the figures were combined to give a mean value for the population for a period of three weeks, and the results are given in Table IV.

For each island there was a control area in which catches were made, so that some measure of seasonal variations of populations might be obtained. As

TABLE III.

Standing catches of *G. palpalis*, expressed as weekly means of non-teneral males caught per station.

Date	Sowe	Kyagwe (control)	Nfo	Musole (control)	Kimi	Mbiru (control)	Tavu
July, 1948	4.2	4.6	5.3	3.4	5.0	7.2	5.3
August	5.2	4.0	5.2	2.5	5.6	5.8	5.2
September	6.3	4.2	5.4	3.1	6.4	6.3	4.9
October	6.2	4.4	6.0	3.1	4.9	5.6	4.9
November	6.5	4.3	6.2	2.0	4.4	4.8	4.6
			1st Application				
	5.9	4.0	6.5	2.6	3.8	4.2	3.4
	6.5	4.0	6.7	2.8	4.4	5.3	4.0
			2nd Application				
	6.7	4.6	4.4	3.8	4.2	5.1	4.4
	5.0	3.4	6.2	2.9	4.3	5.0	4.3
			3rd Application				
	5.6	—	6.0	2.3	4.0	—	3.4
	6.1	3.4	—	4.0	3.4	5.8	3.6
			4th Application				
	4.4	3.9	5.1	3.6	4.0	5.5	4.0
	3.5	4.3	5.9	3.9	4.1	6.1	3.5
			5th Application				
	5.2	4.2	5.1	4.2	4.4	6.2	4.8
	5.9	5.3	5.3	3.2	4.1	5.7	5.6
			6th Application				
	4.7	6.1	4.6	3.4	4.5	4.6	5.0
	4.8	5.1	4.1	3.4	—	4.8	4.9
			7th Application				
	5.5	6.1	4.3	4.5	2.5	4.7	5.0
	3.9	4.0	4.5	3.4	2.8	4.9	5.9
			8th Application				
March, 1949	4.4	6.9	3.8	4.7	3.4	3.6	5.1
April	5.6	6.8	4.2	3.5	3.9	5.6	4.6
May	3.6	6.9	3.8	3.6	4.1	4.9	4.6
June	4.2	4.9	4.4	4.2	3.3	8.9	5.0
July	3.5	7.0	4.6	3.7	2.9	9.0	5.0

may be seen from fig. 1, Musole and Kyagwe, the controls for Nfo and Sowe, were very close to their respective islands, but Mbiru, the control for Kimi and Tavu, was less satisfactorily situated.

Correcting the catches for seasonal variation by using the control catches, there was a 20 per cent. reduction in the catches upon Sowe after the first five

TABLE IV.

The estimates of populations of non-teneral male flies, both before and after the series of applications.

Island	Sowe		Nfo		Kimi		Tavu	
Date	August 1948	March 1949	August 1948	March 1949	July 1948	March 1949	July 1948	June 1949
Population	8,870	3,280	4,460	1,390	3,690	3,350	14,560	11,610

applications. The dosages were then increased, and after eight applications the reduction was 40 per cent. The population estimates showed that the population after the applications was 40 per cent. of the original population. On Nfo there was a reduction of 30 per cent. after five applications and a reduction of nearly 60 per cent. after the eight applications; the population after the treatment was 30 per cent. of the pre-treatment figure. On both Kimi and Tavu there were no significant reductions after six applications. The last two applications reduced the catches temporarily, but, since they covered only a very short period, emergences soon re-established the adult population. At the end of the series of applications reductions in catches were 20 per cent. and 10 per cent. for Kimi and Tavu respectively, and the corresponding population estimates gave reductions of 10 per cent. and 20 per cent.

Discussion.

It appears that the aerosols were slowly reducing the fly populations, but the kill for any one application could not have been very high. If the increased dosages of the later applications had been more effective, a temporary increase in reduction would have been obtained which would have disappeared as emergences took place from pupae laid down during the earlier applications. The final reduction in population, however, was maintained for four months after the last application, and this can only be explained if the increased dosages did not greatly increase the kill. The first six applications of emulsions, in the form of sprays, did not significantly affect the fly populations; the last two applications, in which increased dosages were laid in dense strips, did cause some reduction, but this was only temporary.

With the emulsions it was partly the inability of the large drops to penetrate to fly haunts that was responsible for the insignificant kills, but it is also most probable that the nominal dosages used were quite inadequate to produce a lethal deposit upon the vegetation. Other work (Symes & others, 1948) in which the vegetation was sprayed by ground methods, showed that the insecticide must be applied to selected areas at rates of approximately 10 lb. per acre for an appreciable reduction in fly population to result.

Even the aerosols were relatively ineffective in producing adequate concentrations within the canopy. As will be shown in later papers, the larger droplets were filtered out in the upper layers of vegetation, and the smaller droplets were so dependent upon the meteorological conditions that they failed to reach in appreciable numbers the lower levels where the tsetse flies lived.

In areas such as are inhabited by *G. palpalis*, with their high and dense canopies, a great wastage of insecticide is inevitable if the solutions or emulsions are applied from aircraft. The dosages used in these experiments were too small to produce any effective reduction in fly populations, and it is certain that they would have to be greatly increased to produce useful kills. The costs of applying such dosages from aircraft would be prohibitively high, and the money would almost certainly be better spent in developing ground methods for applications in such areas.

Significant kills were obtained only with the aerosols. Although this does not conclusively show that the fine droplets are more effective than the spray drops in all types of vegetation, it does suggest that the aerosols would give greater kills, particularly when it is remembered that at the dosages used it is improbable that lethal residual deposits can be produced. In most of our later work aerosols were used against *G. morsitans*, *G. swynnertoni*, and *G. pallidipes*, and very high kills were in fact obtained.

Summary.

Preliminary experiments are described of applications from aircraft of coarse sprays and coarse aerosols. The experiments were carried out over dense forest areas containing the tsetse fly *G. palpalis*.

At dosages of 0.2 lb. per acre of the p,p' isomer of DDT, or 0.032 lb. per acre of the γ isomer of BHC, both the sprays and the aerosols were relatively ineffective, and significant kills were obtained only with the aerosols.

The sprays were ineffective not only because they did not penetrate the canopy, but also because the nominal dosage was in any case too small to produce lethal deposits upon vegetation.

Much of the aerosol was filtered out by the upper layers of the canopy. The meteorological conditions in the area were also unsuitable for the application of aerosols, and much of the insecticide did not reach the canopy, but was blown away from the treated area.

It is concluded that aircraft applications of insecticide against *G. palpalis* are wasteful of insecticide, and would be very costly if substantial reductions in fly population were to be obtained. If insecticides are to be of value in such areas, ground methods of applying them would be almost certainly more effective, and less costly.

Acknowledgements.

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References.

- GUNN, D. L. & others. (1948a). Locust control by aircraft in Tanganyika. 153 pp. London, Anti-Locust Res. Cent.
GUNN, D. L. & others. (1948b). Anti-Locust Bull., no. 4, 121 pp.
JACKSON, C. H. N. (1944). Ann. Eugen., **12**, pp. 176-205.
MAY, K. R. (1945). J. sci. Instrum., **22**, pp. 187-195.
SYMES, C. B., HADAWAY, A. B., BARLOW, F. & GALLEY, W. (1948). Bull. ent. Res., **38**, pp. 591-612.
DU TOIT, R. & KLUGE, E. B. (1947). Vet. Rec., **59**, pp. 569-574.
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AIRCRAFT APPLICATIONS OF INSECTICIDES IN EAST AFRICA.

II.—AN EXPERIMENTAL ATTEMPT TO PRODUCE A FLY-FREE CORRIDOR THROUGH A BELT OF TSETSE-INFESTED WOODLAND.

By K. S. HOCKING, H. C. M. PARR, D. YEO and P. A. ROBINS.

L.C.

Colonial Insecticide Research Unit, Arusha, Tanganyika.

In certain areas of tropical Africa there is a large seasonal movement of live-stock in search of grazing. The numbers of animals involved in this movement are large, and it often happens that grazing areas are separated by belts of woodland infested with tsetse flies. Large numbers of cattle also move along well-defined stock routes from the main cattle-rearing areas to distant centres of population, and in many cases these stock routes also pass through tsetse belts. Livestock is thus exposed to the risk of infection with trypanosomes, and it would be useful to be able to maintain fly-free corridors through belts of infested woodland, if only for limited periods of the year.

An experimental attempt was made to produce such a corridor by applying from aircraft a coarse spray of an oil solution of DDT. Later work has shown that a coarse aerosol would have been more suitable than a coarse spray, but nevertheless it seems worth while to record the experiment.

Description of the Area.

A section of the cattle route from Sukumaland, south of Lake Victoria, was chosen for the experiment. Along this route thousands of cattle and other live-stock pass every month to the slaughterhouses of the Northern Province of Tanganyika and the Nairobi area of Kenya. Infection of livestock with trypanosomes was not so important in this case, since the animals were quickly slaughtered at the end of their journey, but the conditions, it was thought, simulated closely those which often apply to large seasonal movements of live-stock, and the area was conveniently near the adequate aerodrome at Arusha, Tanganyika.

The treated area was two miles wide, centred on the stock route, and was four miles long. It was near Mt. Essimigor, and at Lat.—03°28'S., Long.—36°10'E.; this is approximately 30 miles south-west from Arusha, where the aircraft were based. An approximate map of the area is given in fig. 1. It formed part of the northern edge of a belt of woodland supporting the tsetse fly *Glossina swynnertoni* Aust., and was mainly covered with light deciduous scrub of *Acacia* and *Commiphora* spp., although there were many open, grassy, clearings. The country was undulating and crossed by many ridges, and was at an altitude of about 4,000 ft. above sea level. In the dry, ravine-like, rivercourses there was dense deciduous thicket, most of which was in full leaf during the applications. Along the north-western edge there was a large, open, "mbuga" (grassland), and in this direction generally the country was open. To the south-west, the direction from which the livestock mostly came, the country was mostly open, with occasional patches of scrub. To the north-east there was very light woodland, which contained very few flies, and the only truly continuous woodland extended to the south-east of the treated area.

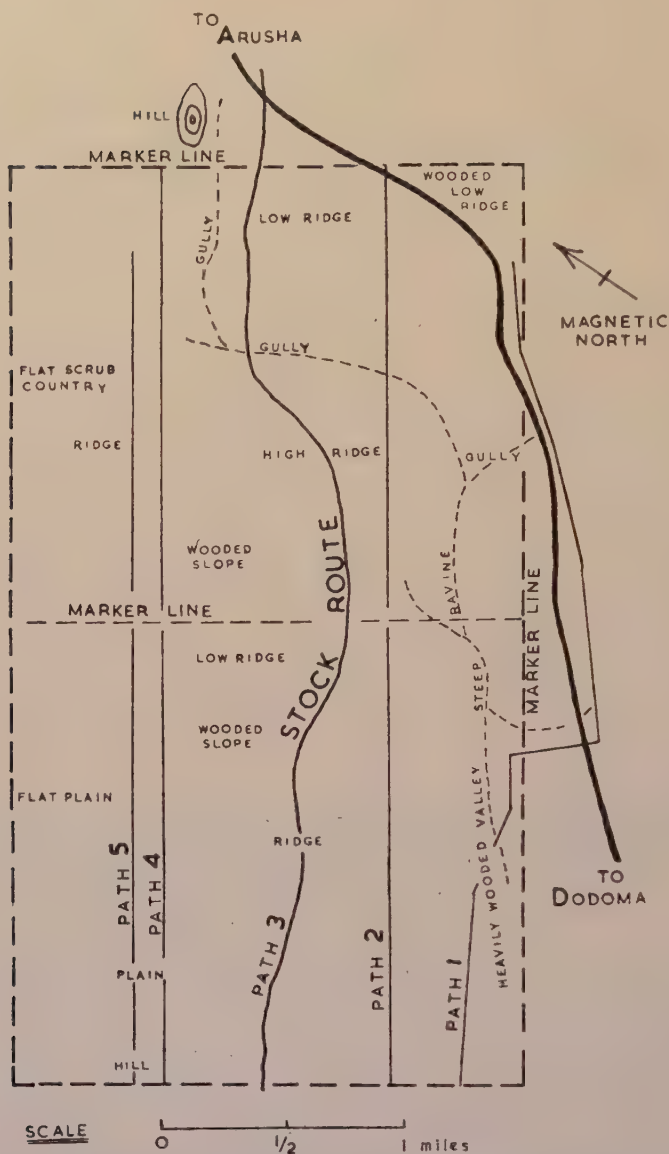


Fig. 1.—An approximate map of the treated area, showing the fly paths, the main vegetation communities and certain topographical features.

The Control of Dosage and the Spraying Operations.

Nominally a 5 per cent. w/v solution of technical DDT (80 per cent. p,p'isomer) was used, with Shell Diesoline as the solvent. It was prepared by heating the Diesoline to approximately 105°F. and then adding the DDT powder. Due to an unusually high proportion of insoluble material in the DDT powder a 4.6 per cent. w/v solution was actually obtained. This was applied at a nominal dosage of 0.5 gallons per acre, giving a nominal dosage of the p,p'isomer of DDT of 0.185 lb. per acre. For reasons given in the first paper of this series

(p. 589), seven applications were made, at intervals of approximately two weeks, so that the applications covered two pupal periods.

The spray was applied using the swathing technique developed at Porton, England, as described by Gunn and others (1948a, 1948b). The aircraft installation for producing the spray has also been described by Hocking and Yeo (*loc. cit.*) and Gunn and others (1948b). It was not possible to determine the drop spectrum and ground recovery until after the series of applications had been completed; using previous experience, a height-wind product of 2,000 (ft. \times m.p.h.) and a swathe width of 88 yards were chosen. The aircraft airspeed was 120 m.p.h., so that the required emission rate was 0.53 gallons per second. The appropriate wind speed to use in the height-wind product is the vector mean of the wind velocity from ground level to the aircraft height, along a direction at right angles to the aircraft track. Gunn and others (1948b) found it to be sufficiently accurate to use the value of the wind velocity at either 100 ft. or 200 ft., as measured by means of smoke puffs and clinometers. Supplies of smoke puffs were insufficient to use this as a regular method in the present work. Instead, a "three-course-drift" determination of the wind speed and direction at 200–300 ft. was made by the aircrew; the wind speed and direction at 6 ft. was measured by an observer on the ground. The mean of these two measurements was used to calculate the spraying height. A few determinations with smoke puffs showed that only rarely did the "three-course-drift" give a result that was seriously in error in magnitude, although some errors in direction often occurred. Usually, therefore, the wind direction was considered to be that shown by the drift of smoke from a small fire.

Aircraft heights were measured with a balloon theodolite, and were rarely more than 20 per cent. in error at the beginning of a spraying run. Indeed, although the ground undulated considerably, the heights flown were a good approximation to the required heights, except near the ridges. It was of some help to the pilot, for judging height, first to reset his altimeter during a low run over the area.

During the first few sorties the required flying track was obtained in the following manner. A preliminary run was made over two smoke markers placed on the ground along the required bearing; during this run the pilot set the compass in the aircraft. The rest of the runs of a sortie were then done on this corrected compass course. During the first application, however, a simpler and more accurate method was evolved. The aircraft was flown over the ground control point, as nearly as possible along the required track; the observer on the ground measured the actual track, and signalled it to the pilot, who was then able to reset his compass to give the required track.

Wind observations were taken for a few weeks before the experiment started, and they showed that the wind generally blew from the north-east. Permanent marker posts were therefore placed along the south-eastern edge of the treated area, at intervals of 88 yds. and along a bearing of 235°M. These were used during the first two applications, each run being indicated by a smoke generator which moved along the marker line between runs. During the third application it became evident that the south-east monsoon had begun to dominate the wind which then blew from the south-east. Two more marker lines were therefore laid out, each along a bearing of 325°M., one along the north-eastern edge and the other across the centre of the area. These markers were used for the subsequent applications, and the area was treated as two blocks, each 2 \times 2 miles, and separated by the central marker line.

Each aircraft normally carried 140 gallons of insecticidal solution, enough to treat 280 acres. One aircraft was therefore able to make four runs, each two miles long, and cover an area approximately 350 yds. wide; a sortie with two aircraft flying in formation treated an area approximately 700 yds. wide. During the first three applications sorties were only done during the early morning and

late afternoon, to avoid the periods of high atmospheric turbulence, and a complete application took five or six days. The value of the spray in producing a residually toxic deposit was doubtful, and since the last four applications had to be done with only one aircraft, it was felt that this slow rate of progression might allow many flies to escape destruction by moving between sorties from untreated areas to previously treated areas. For the last four applications, therefore, sorties were done throughout the day. It was assumed that the more uneven dosages obtained during the turbulent periods of the day would be more than compensated for by the reduction in the time between sorties and the consequent reduction of the number of flies escaping the spray because of their movement.

Although two aircraft were used for the earlier applications, one aircraft would have been sufficient to treat the entire area. In fact, the last three applications were completed with ease with only one aircraft; the other aircraft was then receiving its yearly intensive inspection.

Physical and Chemical Assessments.

No dye was available during the series of the applications. Shortly afterwards, however, a supply of Waxoline Red oil-soluble dye was obtained, and the drop spectrum of the spray, and the ground recovery, were determined. The arrangement of the assessment points was similar to that used by Gunn and others (1948a), deposits were estimated colorimetrically, using a Spekker absorptiometer, and the densities of the number of droplets in the deposit were obtained from counts of stains upon absorbent paper. In this experiment the aircraft height was 100 ft., the aircraft airspeed was 120 m.p.h.; from each emission pipe 0.5 gallons per second were emitted, and both pipes were used so that a large

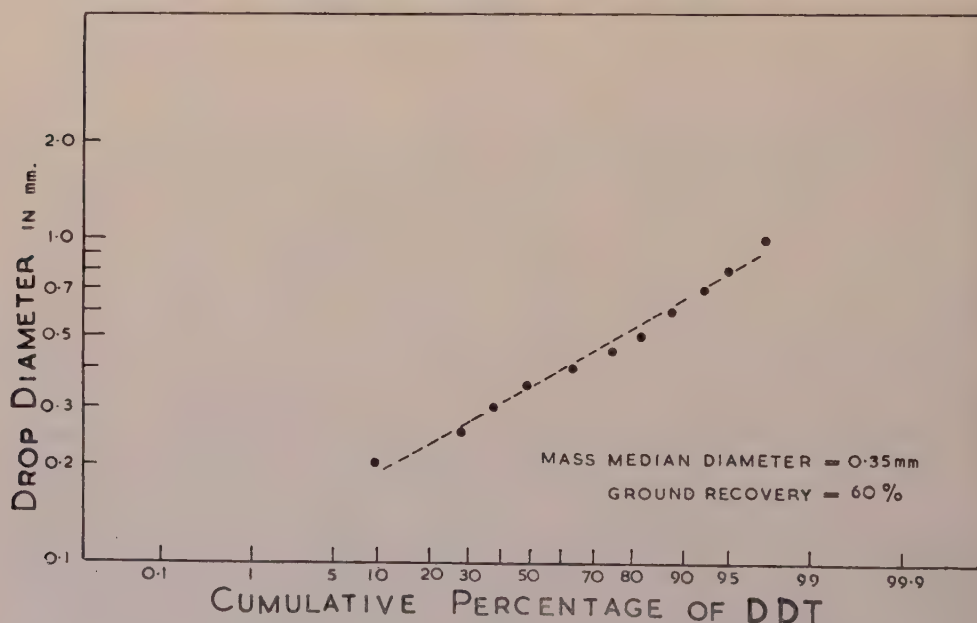


Fig. 2.—The drop spectrum of the ground deposit, plotted on log-probit paper.

ground deposit was obtained. At 6 ft. above the ground the air temperature was 88°F., and the wind speed 5 m.p.h. The drop spectrum of the ground deposit, plotted on log probit paper, is shown in fig. 2; the mass median diameter was 0.35 mm., and the ground recovery was 60 per cent.

Using this drop spectrum and the ground recovery, and the terminal velocities of the drops, it was possible to calculate the theoretical distribution of the ground deposit for a given height-wind product and swathe width. The average ground dosage was 0.3 gallons per acre, *i.e.*, 60 per cent. of the nominal dosage, but this dosage was built up from deposits produced by several runs. The theoretical distributions are shown in fig. 3. Graph (a) shows the theoretical deposits laid down during sorties with one aircraft; the actual dosage during a sortie

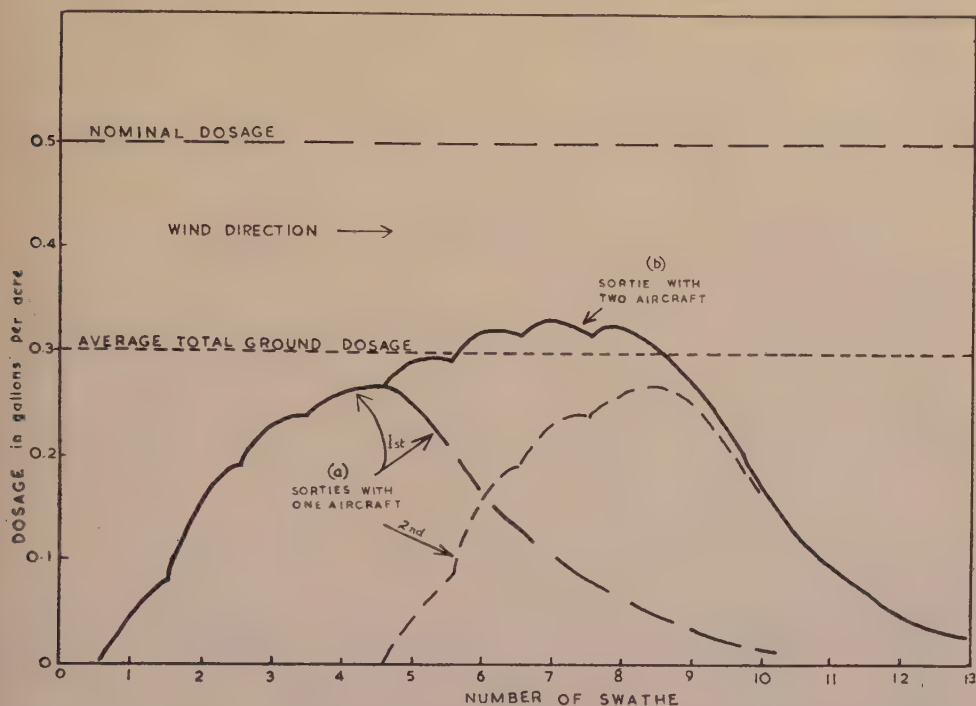


Fig. 3.—The theoretical distributions of the ground dosage. (a) Dosage distribution across the swathes for a sortie with one aircraft. (b) Dosage distribution during a sortie with two aircraft.

with one aircraft was considerably less than the average total dosage. Even during a sortie with two aircraft in formation, in which eight runs were made, nearly 20 per cent. of the area to be covered by the sortie, that lying on the upwind side, received dosages less than 75 per cent. of the total average dosage, as may be seen from graph (b) of fig. 3. In fact all swathes except that on the upwind side of the first sortie received deposits from more than one run, and all areas, after that covered by the first sortie, received deposits from more than one sortie. It was only the total average ground dosage that was 0.3 gallons per acre.

The dosage distributions shown by graphs (a) and (b) in fig. 3 apply to areas near the markers, at the beginning of each of the runs. The emission rate fell continuously as the storage tanks emptied, and dosages at the ends of the runs at points farthest from the markers were in general 15 per cent. lower than those given in fig. 3.

During the applications the dosages of DDT were estimated directly. The deposits were dissolved in petroleum ether, and the DDT was hydrolysed with 2 per cent. alcoholic potash. The chloride ion was estimated by an ~~an~~electrometric

the

Volhard titration method, using $N/10$ potassium thiocyanate. Rows of sampling papers, laid out at right angles to the line of flight, showed that random variations in dosage far exceeded the small variations due to swathing. A variation in dosage of 5 times was common, and on some occasions the variation was as much as 20 times. Average recoveries of DDT from open sites varied from 50 to 80 per cent. and thus agreed quite well with the recovery of 60 per cent. obtained during the drop spectrum determination.

Attempts were made to assess the effect of vegetation cover upon the distribution of the insecticide. There was no continuous canopy. Representative trees were studied by placing a mosaic of sampling papers near and underneath them. Individual samples varied enormously, and in this type of savannah woodland it was quite impossible to find any "shadows" of consistently low dosage. Under the trees dosages varied from 20 to 180 per cent., and averaged 80 per cent., of the mean dosage of areas near by. Freak effects occurred; for example, the apparently leeward side of the trunk of a tree had received a dose upon one occasion. In general, however, the leeward sides of obstacles did not receive a dose and no deposits were found on the under sides of objects.

Assessments were made at the ends of the runs farthest from the markers, primarily to check whether errors in tracking had any serious effect upon the dosage distribution. On the three occasions when such assessments were made, however, the tracking was remarkably good, and dosages and swathe spacings were as consistent as at the marker. Such extreme accuracy of flying was not always obtained; on some occasions the tracks of the two aircraft crossed. It is most improbable, however, that any area received a greatly reduced dosage because of tracking errors, particularly since the spray from one run was spread over such a relatively wide area.

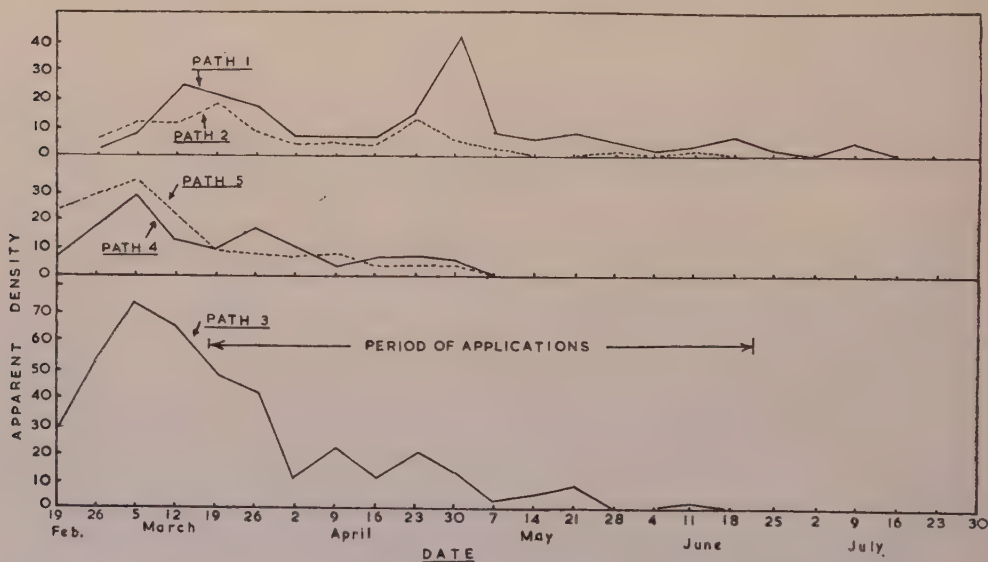


Fig. 4.—The fly catches on the five paths during the period covered by the applications.

Entomological Observations.

Only the species *G. swynnertoni* was found in the area. The fly population was studied upon the five fly paths shown in fig. 1. Path 3 followed the stock route itself. Paths 2 and 4 were roughly parallel to the stock route and about half a mile from it; Path 5 followed approximately the north-western edge of the

woodland, while Path 1 was approximately 1 mile south-east from the stock route, and much of it was outside the treated area. Each path was divided into sections 100 yds. long, and catches were made in the usual fashion by two African assistants (see Swynnerton, 1936), who stopped at least once in each section of 100 yds. to catch all the flies near them. The catches are given in fig. 4 and summarised in Table I, and are recorded in terms of the apparent density, *i.e.*, the number of non-teneral male flies caught per 10,000 yds. of fly path (see Swynnerton, *op. cit.*). During the period of the applications catches were made as soon as possible after each application and during the intervening week.

If the treated area had been part of an extensive area of infested woodland, in which the fly population, normally stable, had supported itself by breeding, the catches over much of Path 1 should not have been greatly affected by the treatments. To an extent depending upon the effectiveness of the applications, and the degree of infiltration into the area, catches upon Paths 2, 4 and 5 should

TABLE I.

Monthly summaries of the catches of non-teneral male flies, expressed as apparent densities.

Fly path	Before spraying	During the applications			After the applications	
1	14.1	9.3	17.4	4.8	1.8	0
2	12.5	7.1	1.9	0.7	0	0
3	51.5	16.4	7.6	0.4	0	1.0
4	14.3	5.3	1.4	0	0	0
5	23.5	4.3	0.7	0	0	0

have decreased, and, if the experiments had been successful, no flies should have been caught along the stock route on Path 3. In fact, as may be seen from fig. 4 and Table I, the catches were greatly reduced upon all paths. The reduction was least rapid on Path 1, but even there catches fell to a very low figure by the end of the treatment, and no flies were caught there during the second month after the end of the applications.

Subsequent catches upon the stock route showed that in the fourth month after treatment the fly population had begun to rise quite rapidly, and by the end of the fifth month catches were approaching their pre-treatment level. Unfortunately these later figures were lost when the headquarters of the unit were transferred from Uganda to Tanganyika.

Discussion.

It is difficult to explain satisfactorily the way in which the catches fell during the experiment. An analysis of the catches during the period of the first four applications suggests that the average kill per application was approximately 70 per cent. From a theoretical point of view, and from results in other work, such a relatively low kill, carried on for two pupal periods, would only reduce a stable, self-contained, population to about 10 per cent. of its pre-treatment level. Yet in this experiment the flies had been very nearly eliminated by the end of the second pupal period.

The structure of the fly population was probably complicated by the presence of immigrant flies. Although the country was open to the south-east, there is little doubt that flies could have been brought into the treated area by the 4,000 animals that were driven along the stock route each month. Such infiltration would, however, reduce the effect of a 70 per cent. kill, since the number of immigrant flies would not be affected by the applications whereas the pupal population and the number of emergences later in the applications would be

reduced. It is not therefore possible to explain the very large reduction that occurred except by assuming that both breeding and immigration had reached a very low level during the latter part of the experiment and for the subsequent two months, or that infiltration was slight and that the later applications caused a much higher kill than 70 per cent.

Measurements of temperature and relative humidity were made only during the aircraft sorties. An examination of the meteorological data collected between 0900 hr. and 1100 hr. suggests that over the months covered by the applications the air temperature dropped from 77°F. to 66°F., and the relative humidity rose from 55 to 65 per cent. This variation in conditions would not normally be accompanied by a large decrease in breeding; indeed, this period of the year is normally associated with an increase in population density. Thus there is no support for the suggestion that breeding fell during the later applications.

The reduction in catches upon Path 1, much of which was outside the treated area, does, however, suggest that there at any rate breeding was on a negligible scale after the fourth application and that the population was normally maintained from the stock route itself. There was no obvious difference between this area and the treated area, so it seems likely that breeding was nowhere very extensive, and that the population over the entire area was maintained principally by immigrant flies. To explain the results, it is then necessary to assume that immigration had fallen to an extremely low level by the end of the applications, and remained extremely slight for the following two months. The numbers of cattle passing along the stock route increased during the period of the applications, however, so that the number of immigrants would not have been expected to fall although it is possible that the number of flies being carried into the area was lower during the dry season following the applications.

The later applications might have been more effective, although there is little evidence that they were. They included many sorties during adverse meteorological conditions, and dosages were almost certainly more variable during such treatments. On the other hand, leaf cover decreased slightly during the series, and it is also true that escapes by movement would probably have been fewer during the latter part of the series, but it seems improbable that these factors made a marked difference to the kills.

There is therefore no entirely satisfactory explanation of the very high reduction in fly numbers. It seems likely that breeding was never very extensive in the area, and that the population was normally maintained largely by immigrant flies, the numbers of which fell off for some reason towards the end of the series of the applications, remained low during the subsequent two months, and then rose again.

The final reduction in fly numbers is not therefore of much value in predicting the reductions that could be obtained in areas supporting stable, self-supporting, populations. It is fairly certain, however, that the average kill per application was not much greater than 70 per cent. and this would not produce an adequate reduction under most circumstances, unless the applications were continued for several pupal periods. This would be uneconomical, particularly since later work has shown that when a coarse aerosol, with droplets varying in diameter from 5 microns to 250 microns, is used in savannah woodland at the same nominal dosage as was used in this experiment, kills per application are of the order of 95 per cent. With these aerosols applications covering one pupal period might produce a sufficiently high reduction in fly population for cattle to move safely through the area.

The recoveries of insecticides are in good agreement with previous work. It seems likely that the 40 per cent. or so of insecticide that is normally lost when coarse sprays are applied from aircraft is contained in small droplets, whose movements, being greatly affected by atmospheric eddies, cannot be predicted

from a knowledge of the terminal velocities of the droplets and the mean wind speed. The fact that no dosages were obtained upon the under sides of obstacles, or on leeward surfaces except under freak circumstances, is also in accord with other work, both theoretical and practical.

The random variations in dosage far exceeded the small variations due to swathing. This was mainly caused by atmospheric turbulence, but partly because the height-wind product was rather large. However, it is usually possible to be considerably in error in estimating the height to be flown, and yet still have a considerable overlap between successive runs. Errors in tracking can have a greater effect upon the dosages distribution than errors in height, and it is important that the tracking of the aircraft should be as accurate as possible. For most applications in woodland it is only possible to indicate to the pilot the start of each run, so that much is left to the skill of the pilot. With experience, a good pilot seems capable of flying much more accurately than would be expected from a study of his instruments. From sortie to sortie there is often a degree or so between two tracks that should have been the same, but during a particular sortie it is rare for successive tracks to differ by more than $\frac{1}{2}^\circ$, providing that the wind conditions do not change considerably. In fact the pilots seem to use an excellent combination of flying by instruments and upon visual reference points.

Summary.

An experimental attempt was made to produce a fly-free corridor through a belt of savannah woodland containing the tsetse fly *G. swynnertoni*.

An area two miles wide and four miles long was treated with a coarse spray of a 4.6 per cent. w/v solution of DDT in Shell Diesoline. The dosage per application was 0.5 gallons per acre, and seven applications were made, at intervals of approximately two weeks, so that the treatment covered two pupal periods.

The fly density had fallen to a very low level by the end of the experiment, and the area remained virtually free from flies for the subsequent two months. An examination of the data suggests, however, that the fly population was maintained largely by immigrant flies, and was certainly subject to wide variations, and it seems certain that the effect of the applications would have been considerably less upon a stable, self-supporting population.

The drop spectrum of the ground deposit had a mass median diameter of 0.35 mm., and the recovery of insecticide in the area was approximately 60 per cent. Leeward and under sides of obstacles did not receive a dose although in some cases dosages were obtained on apparently leeward sides, probably because of local reversals of wind direction.

Acknowledgements.

We are grateful to the staff of Messrs. Airwork Ltd., for the excellent flying. We should also like to thank Mrs. J. E. Yeo for her assistance during a period when there was a shortage of European staff. Other members of the unit helped in the field and assessment work, notably Messrs. G. C. Dauber, E. T. Mesmer and H. V. Newton.

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References.

- GUNN, D. L. & others (1948a). *Anti-Locust Bull.*, no. 4, 121 pp.
 GUNN, D. L. & others (1948b). *Locust control by aircraft in Tanganyika.*—
 153 pp., London, Anti-Locust Res. Cent.
 HOCKING, K. S. & YEO, D. (1953). *Bull. ent. Res.*, **44**, pp. 589-600.
 SWYNNERTON, C. F. M. (1936). *Trans. R. ent. Soc. Lond.*, **84**, pp. 1-579.

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AIRCRAFT APPLICATIONS OF INSECTICIDES IN EAST AFRICA.

III.—ATMOSPHERIC TURBULENCE IN WOODLAND.

By B. W. THOMPSON.

Colonial Insecticide Research Unit, Arusha, Tanganyika.

In aerosol applications against species of *Glossina* the degree of atmospheric turbulence between the aircraft and the top of the bush largely determines the proportion of insecticide which will be able to penetrate the canopy. A secondary problem, however, concerns the ultimate distribution of such insecticide as has penetrated. Since the diameters of the droplets found to be most effective are of the order 10–100 microns they are to a large extent airborne, and their distribution and diffusion is much controlled by turbulent motion within the bush. An attempt was therefore made to ascertain the nature of air motion within tsetse bush and to determine the most suitable times of day for insecticidal application from this point of view. Since the various species of *Glossina* inhabit all types of woodland from thin dry savannah bush to dense forest or thicket it is quite impossible to investigate all variations, and the experiment was confined to a conveniently situated block of natural woodland of a density that could support a high tsetse population. It was thought that the investigation would also be valuable in that it would throw light on the dissemination to be expected of insecticide whose source was initially within woodland, whether natural or plantation, and would have a wider application than the mere case of anti-tsetse operations by aerosol applications from aircraft.

Some Notes on Atmospheric Turbulence and its Assessment.

In the free atmosphere two distinctly different states of air motion can readily be recognised. The first is a turbulent atmosphere characterised by wind gustiness, i.e., eddy motion of air; the second is a non-turbulent atmosphere manifested by quiet conditions and smooth airflows. The nature of the motion of air in the lower layers of the atmosphere is determined chiefly by frictional and thermal influences. The former is self-evident; friction may cause an overturning of the moving air immediately in contact with the ground, and the resulting disruption or eddying will naturally tend to spread into higher layers of the atmosphere. Such frictional eddying is greater the stronger the winds and the more uneven the nature of the ground. The thermal effect acts through changes in the density of air in contact with a heated or cooled ground, differentially heated air tending to rise by convection, cooled air tending to sink or become stagnant. For a fuller account of atmospheric turbulence, Sutton (1949) should be consulted.

The atmospheric state of neutral vertical equilibrium is that state in which a parcel of air, forced to ascend or descend, always has the density of its environment; it thus has no internal convective or slumping volition. The temperature structure, or lapse rate, of such an atmosphere (when unsaturated) is the dry adiabatic lapse rate, i.e., a fall of temperature with height of 1°C. per 100 metres (approximately 0.1°F. per 7.5 m. to refer to the values used in this paper).

If a particular parcel of air develops a lapse rate of more than $1^{\circ}\text{C. per } 100 \text{ m.}$ it is said to have super-adiabatic lapse rate and to be unstable. Such a lapse rate develops in a mass of air when the lower layers are warmed by contact with a heated ground. The heated air, less dense than unheated or less-heated surrounding air, will rise convectively and will continue to rise until density adjustment with its environment is eventually reached. If however a particular mass of air has a temperature fall with altitude of less than $1^{\circ}\text{C. per } 100 \text{ m.}$, or, in the majority of cases, shows a temperature increase with height (a negative lapse rate), an inversion of temperature is said to exist. This, in meteorological circles, is invariably called an "inversion". A particular case is that of temperature constant with height, termed an "isothermal" atmosphere. These are the conditions of stability, and usually develop when the lowest layers of air are cooled by contact with a cold ground; since in this case air density decreases with height there is resistance to upward motion. This is generally the quiet non-turbulent condition of the atmosphere. In short, instability exists and convection develops with super-adiabatic lapse rates, and stability, with stagnation or slumping motion, tends to develop with inversions or isothermal conditions. It is evident that frictional over-turning will be intensified when the temperature structure is unstable and damped when conditions are stable. A practical study of atmospheric turbulence thus involves the measurement of both wind speed and lapse rate of the atmosphere.

The most common condition to be anticipated in the free tropical atmosphere is one of considerable turbulence by day when the ground is strongly heated, and little turbulence by night when the ground is cooled. Indeed, by night, an approach towards smooth, non-turbulent flow would be expected in many areas since under strong inversions ground surface irregularities may be much smoothed by the development of pools of stagnant cold air in hollows, over which upper air, especially when of low velocity, may slide with little frictional disturbance. Such smooth airflows often have a laminar structure and are termed "laminar airflows".

A turbulent atmosphere is manifested by wind gustiness; wide fluctuations in speed and direction occur continually. The air motion may be resolved into components in the direction of the mean wind, and in vertical and horizontal planes across the mean wind. The latter pair of components may be studied by the employment of an instrument due to Taylor (1927) called a "bi-directional wind vane". This instrument and the manner of its use have been extensively described by Scrase (1930) and Best (1935), both a diagram and photograph to illustrate the manner of its construction being contained in the latter. It is a delicate instrument consisting of a rod bearing at one end horizontally and vertically mounted aerofoil-shaped vanes and at the other end an adjustable balance weight. This rod is universally jointed to a vertical support and is thus free to register combinations of vertical and horizontally swinging air motion. A light rod carrying a pen at its lower end is pivoted, on the vanes side of the universal joint, to the rod carrying the vanes. The pen traces the motion of the vanes (and hence the vertical and lateral components of the air motion) on a rectangular chart held in a semi-circle of suitable radius at the base of the instrument. The length of the pen-arm is so arranged that when the vane is swinging in a horizontal plane the pen traces a straight horizontal line across the middle of the chart. Absolutely steady air motion would result in only a point trace on the chart since there would be no motion of the vane. If such air motion were downwards the point would be below the equator-line of the chart, and if upwards above it. A swinging motion in a vertical plane would result in a vertical line on the chart. There are, of course, many combinations which may occur and, in particular, when the atmosphere is very turbulent traces may be very erratic, although in the free air they conform to an elliptical pattern. It

will be seen that the degree of atmospheric turbulence may be roughly classified according to the nature of the trace on the chart.

There appears to have been little previous published work on the precise nature of atmospheric turbulence within woodland, although related aspects of the subject have been studied separately.

Geiger (1950) records that by night in a fairly dense stand of trees, "either the whole air mass is isothermal or, if the crown canopy is sufficiently dense, the cold air remains above". In light stands, however, "the sinking cold air of the crown space results in a temperature minimum on the forest floor". During the day maximum temperatures occur in the crown space with, in light stands, a secondary maximum at the forest floor. Variations in conditions according to forest density are thus particularly emphasised, as is the importance of the crown space, or canopy, as the absorber and radiator of heat. Geiger also gives a diagram due to R. Kanitscheider showing the zones of turbulence as measured by "temperature unrest" in a 2.5 m. high growth of young pine. Unrest is at a maximum on the top of the plant cover, decreases very rapidly just below this level, and very slowly within the trunk space. With regard to wind, Geiger records that speeds within the trunk space are generally light and remarkably uniform with height in conditions of light free air winds, but greater variations are found the stronger the winds outside. The importance of outside wind speed in creating turbulent conditions within woodland is thus emphasised.

Hales (1949) has described wind and temperature observations made in what would appear to have been dense tropical jungle in Panama. Here it was also discovered that the canopy acted as the chief absorber and radiator of heat, with the result that during the day temperatures were inversions beneath it and a super-adiabatic lapse rate existed above. By night the opposite was true, an inversion developed in the atmosphere above the canopy and, owing to greater cooling of the canopy by radiation than of the ground beneath, a super-adiabatic lapse rate developed within the forest, and is quoted as being on the average about twice the dry adiabatic value. It is unlikely, however, that the unstable condition created by this nocturnal super-adiabatic lapse rate beneath the canopy could persist indefinitely. Indeed, it would be expected that turbulence should develop as a result of it and persist until a state of neutral equilibrium had been reached. From this point the super-adiabatic lapse would again commence to develop and the whole process would be repetitive.

Conditions within most tsetse bush are not likely to be simple since in general the canopy is neither thick nor continuous, particularly during the leafless period most suitable for aerial spraying, and there must be an interplay between heating and cooling of both canopy and ground surface.

The Site and Methods of Observation.

Observations were made during July and September 1951, in an extensive block of natural and little-disturbed woodland comprising chiefly *Acacia xanthophloea* with scattered *Acacia usambarensis*. The specific site for the instruments was chosen for its good uniformity of tree density and canopy height, the general canopy top (*A. xanthophloea*) being at 19-20 m., the only slight excrescence above this level being an almost leafless *A. usambarensis* which was converted into a ladder from which above-canopy observations could be taken. It is difficult to specify precisely the canopy density. Most of the typically very small leaves were confined to the tops of the trees, giving a thin, spreading, moderately dense canopy through which sunlight filtered on to some 30-40 per cent. of the ground. Undergrowth varied considerably but averaged 1-2 m. in height and was, generally, difficult to penetrate.

A sturdy, open, wooden tower was constructed on which platforms gave access

to instruments mounted at 2.5, 5 and 10 m., rapid and frequent observations in the lower half of the woodland being thus possible. A few yards away grew the convenient *A. usambarensis*, to the trunk of which rungs were firmly nailed so that it could be ascended to the level of the canopy, and it was possible to hold a psychrometer above this, and to hoist a light pole carrying an anemometer to 5 m. above it.

About a mile west of the tower the bush gave way to an open stretch of plains on which is located Arusha airfield, where another tower had been constructed on which simultaneous observations were made when necessary, the distance between the towers being two miles.

Observations of wind and temperature at three heights on the towers were made to a strict routine at half-hourly intervals from 07.00 to 18.30 hours East African Standard Time during each day of July 1951. Cup anemometers (Sheppard pattern) were employed and temperatures were measured by Assmann psychrometers read to the nearest 0.1°F. At $H - 5$ minutes the three anemometers at each site were started simultaneously and at $H + 5$ minutes were stopped, each wind speed recorded therefore being the mean for a 10-minute run. Temperature was somewhat more difficult. The psychrometers were aspirated before $H - 5$ minutes and were read, one after the other, by a single individual commencing at this time. Readings were repeated at H and $H + 5$ minutes, or more frequently, the mean of the observations being taken as the final value for that level. The method undoubtedly leads to some inaccuracies, chiefly in the open during the daytime period of super-adiabatic lapse, but after much experiment it was decided that it was the only practicable one with the personnel and equipment available.

The tree ladder used in September was intended to support short-period observations throughout the whole depth of the woodland. Outriggers at arm's length from the trunk carried anemometers and psychrometers. The wind speed values cover periods of two minutes. The psychrometers were read by two observers, one covering the upper four positions and the other the lower four, on two ascents or descents, the final value being considered the mean of the two readings at each level, the reading interval being three minutes at any one level, and the total time to complete the whole series of temperature observations five minutes.

A bi-directional wind vane was also employed on the tower at 10 m., observations being over periods of two minutes, with wind speed measured concurrently.

Observations of Wind.

The results of twelve two-minute observations throughout the whole depth of the bush are given in Table I, where they are compared with simultaneous observations made on the airfield. Results are arranged in descending order of wind speed at 10 m. in the open, regardless of lapse rate.

It is seen from columns E and F that the principal reduction in wind speed is at canopy level, as was recorded by Geiger. The wind at canopy level is, to a good average, about 40 per cent. of that 5 m. above the canopy in all cases where the speed of the latter wind is greater than 6 feet per second (column O, nos. 1-7). On the other hand, this percentage drops to about 20 in lighter winds, whether free air (airfield) conditions are super-adiabatic lapse or inversion. Hence the effect of winds of typical East African strength, above the woodland, is to create light but irregular air movements inside, with a tendency towards a decreasing speed with decrease in height (columns F-L). With light winds outside the bush (Nos. 8-12) there is little air movement inside, but remarkably

TABLE I.
Comparison of wind speeds in the woodland and at the airfield in different lapse rate conditions at the airfield.

No.	Column		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
	Date Sept.	Time hrs.	Arusha Airfield				Berika Woodland										% ratios	O
			T ₁₀ -T _{2.5}	25 m. (calc.)	10 m.	2.5 m.	5 m. above canopy	Canopy level, 20 m.	17.5 m.	15 m.	12.5 m.	10 m.	5 m.	2.5 m.				
			° F.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	f.p.s.	%	%	%	
1	26th	11.30	- 1.3	27.0	24.0	19.4	16.4	6.9	6.3	5.4	4.9	4.2	4.2	4.0	61	76	42	
2	27th	12.00	- 1.7	25.5	22.7	18.3	15.5	6.6	6.4	3.4	4.1	3.9	3.8	4.0	61	76	43	
3	27th	18.00	- 0.1	24.2	20.6	15.3	9.7	5.0	4.5	2.8	3.2	3.6	2.9	2.4	40	54	52	
4	26th	10.00	- 1.3	20.8	18.5	15.1	12.9	4.9	4.4	4.7	4.3	4.0	3.9	3.8	62	77	38	
5	27th	18.30	- 0.1	18.4	15.7	11.4	6.4	2.3	2.1	2.1	1.6	1.7	1.5	1.6	35	47	36	
6	24th	17.30	- 0.7	16.3	14.2	10.9	8.1	3.1	2.8	2.6	2.7	2.4	2.1	1.8	50	65	38	
7	5th	07.30	- 0.5	12.9	11.7	9.8	6.0	2.4	2.2	1.7	1.9	1.6	1.3	1.4	46	56	40	
8	5th	09.00	- 0.7	8.1	7.3	6.2	3.3	0.7	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	41	49	21	
9	28th	07.30	+ 0.2	—	7.0	5.1	3.5	0.8	1.0	<0.5	0.7	<0.5	<0.5	<0.5	—	58	23	
10	5th	08.30	- 0.9	7.8	6.8	5.3	2.1	<0.5	<0.5	0	0	<0.5	<0.5	<0.5	27	35	<23	
11	27th	07.00	+ 1.4	—	6.5	3.2	3.2	0.7	0.5	<0.5	<0.5	0	<0.5	<0.5	—	71	22	
12	28th	06.20	+ 2.4	—	5.1	4.0	3.1	<0.5	0	<0.5	0	<0.5	<0.5	0	—	69	<16	

Column M = $\frac{\text{Wind 25m. above ground in woodland}}{\text{Calculated wind at 25m. at airfield}} \times 100$

Column N = $\frac{\text{Wind 5m. above canopy}}{\text{Wind at 5m. at airfield}} \times 100$

enough, there are few occasions of absolute calm over the two-minute observation periods. During the 10-minute observation periods in July, less than 1 per cent. of observations showed absolute calms at any time of day or night, and there were none at 10 m. It is notable that Hales' (1949) results show no record of zero wind. Evidently there is almost invariably sufficient air movement within bush of this density to cause slow dissemination of air-borne insecticide, however introduced, in addition to that caused by diffusion alone or by the artificial turbulence set up by the insecticide source. Furthermore, winds are rarely so strong that violent dissemination is likely to occur, except of course in clearings where gustiness is enhanced by stronger winds and increased thermal turbulence.

Since it has been found that, on the surface, high recoveries of aerosol are restricted to occasions of inversion and wind speeds less than about 10 f.p.s. (results to be published shortly), it is important to determine the effect of the canopy in reducing the wind speeds within the free air. This has been estimated in two ways, the results being shown in columns M and N of Table I. It is important to notice that in every case except no. 11, where winds were very light, the wind 5 m. above the canopy was less than that at 2.5 m. on the airfield at the same time.

On the assumption that in the open the wind profile remains logarithmic in adiabatic and super-adiabatic conditions to a height of 25 m. (this result was found to be valid between 2.5 and 10 m. in these conditions on the airfield), the winds at this level at the airfield have been calculated from the airfield results in these lapse rate conditions and are given in column B. The ratio of wind 25 m. from the ground in the woodland (*i.e.*, 5 m. above the canopy) to the calculated wind at the same height in the open, expressed as a percentage, has been taken as a measure of the effect of the canopy in reducing the free air wind speeds, and is given in column M. It is remarkable that in the three cases of high wind and strong super-adiabatic lapse rate (nos. 1, 2 and 4) these ratios are so close, *viz.*, 61, 61 and 62 per cent., respectively. In conditions of adiabatic lapse rate, the ratio falls to 40 per cent. in the case of no. 3 (*cf.* nos. 3 and 4) and to 35 per cent. in the case of no. 5 which has generally lighter winds. In conditions of fairly strong super-adiabatic lapse rate but light winds the ratio decreases with decreasing wind speed (nos. 6, 7, 8 and 10). Thus it appears that for practical purposes it may be assumed that the wind 5 m. above a fairly level canopy in super-adiabatic lapse rate conditions is approximately 60 per cent. of that in the open at the same height (*i.e.*, both winds at same height above a level ground surface) when winds are strong, but with the decreased turbulence associated with a lapse rate approaching the adiabatic, and/or with lighter winds in the open, the speed above the bush is only 50–30 per cent. of that at the same height over open ground. This method, however, is not of immediate practical value. It cannot be applied in conditions of inversion when the wind profile is not logarithmic, and the direct measurement of winds at the appropriate height is, of course, hardly feasible in general. Nor is it wise to place too much reliance upon the assumption of a logarithmic increase of wind to a height of 25 m.

Column N therefore endeavours to correlate the winds at 5 m. above both canopy and ground. Naturally no great accuracy is to be expected since the wind 5 m. above the ground varies as the roughness parameter of the surface (these experiments were conducted with short grass), and the height of the woodland becomes another variable. There is the advantage, however, that results in conditions of inversion can be assessed. In any event the ratios can only be so rough that they may be used merely as indicative, but as a coarse measure probably have a fairly wide application. The wind on the airfield at

5 m. is taken as the mean of the readings at 2.5 and 10 m., permissible since the wind gradient over this range is close to the logarithmic. Once again there is a surprising uniformity in the ratios for conditions of strong wind and high super-adiabatic lapse rate at the airfield (nos. 1, 2 and 4), but with the onset of adiabatic conditions the ratio falls, being less with lower wind speed at the airfield (nos. 3 and 5). With fairly strong super-adiabatic lapse rate and light winds the ratio decreases with falling wind speed at the airfield (nos. 6, 7, 8 and 10). It is evident that even in super-adiabatic lapse rate conditions the frictional effect of the irregularities of the canopy is enhanced when the general free air wind speeds are light and decreasing. With inversion conditions the ratio increases again in the reverse way, being higher with lower wind speeds (nos. 9, 11 and 12), possibly due to the submergence of the main frictional elements of the canopy by almost stagnant air, a state remarked upon by Deacon (1949).

The above results must be accepted with much tolerance. To what extent, for instance, it is coincidental that the three cases of high super-adiabatic lapse rate and strong winds in the open gave such exactly uniform values of the ratios chosen is not known. The variables are too many for any high degree of accuracy in this type of work, in particular the spatial and instantaneous variations of wind are considerable, especially when two miles separate the observing points in the bush and in the open. In the case of winds within the lower half of the bush, compared with those at the same level outside, the result of the complete series of daily observations during July over 10-minute periods indicated only a slightly significant correlation. The plot of wind at 10 m. in the bush against that at the same level at the airfield, for 10-minute runs at each site, and the wind direction at the airfield being a constant south-east, is given in fig. 1. The most that can be said is that mean winds are more likely to be stronger in the

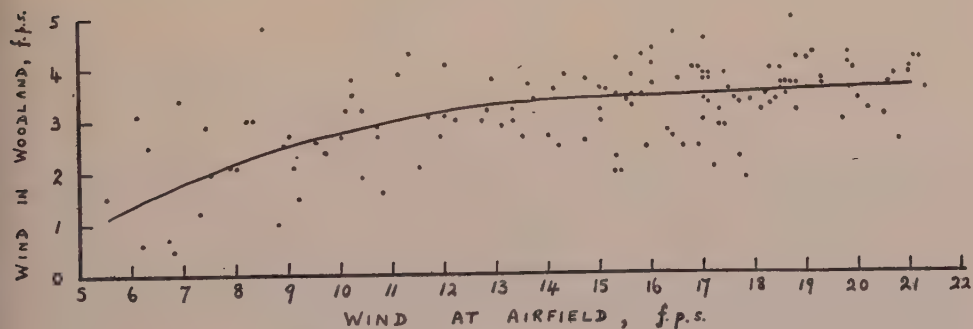


Fig. 1.—Comparison of simultaneous observations at 10 m. of winds within woodland and in the open for 10-minute periods.

bush when they are strong outside. The mean monthly winds for half-hourly intervals during July at 10 m. for the woodland and open are given in fig. 2, from which it is seen that throughout most of the daylight hours, a fairly uniform wind speed slightly greater than 3 f.p.s. was maintained in the bush despite considerable outside variations. The difference in mean wind speed between 10 and 2.5 m. in the bush is also shown in fig. 2. It is interesting that this mean velocity gradient remains sensibly uniform throughout most of the daylight hours, but increases during the evening period of temperature inversion (*cf.* fig. 3).

Observations of Temperature and Lapse Rate.

Instantaneous temperature profiles within the woodland showed very considerable variation from experiment to experiment, despite mean values of lapse rate between 2.5 and 10 m. over 10-minute periods being very constant and close

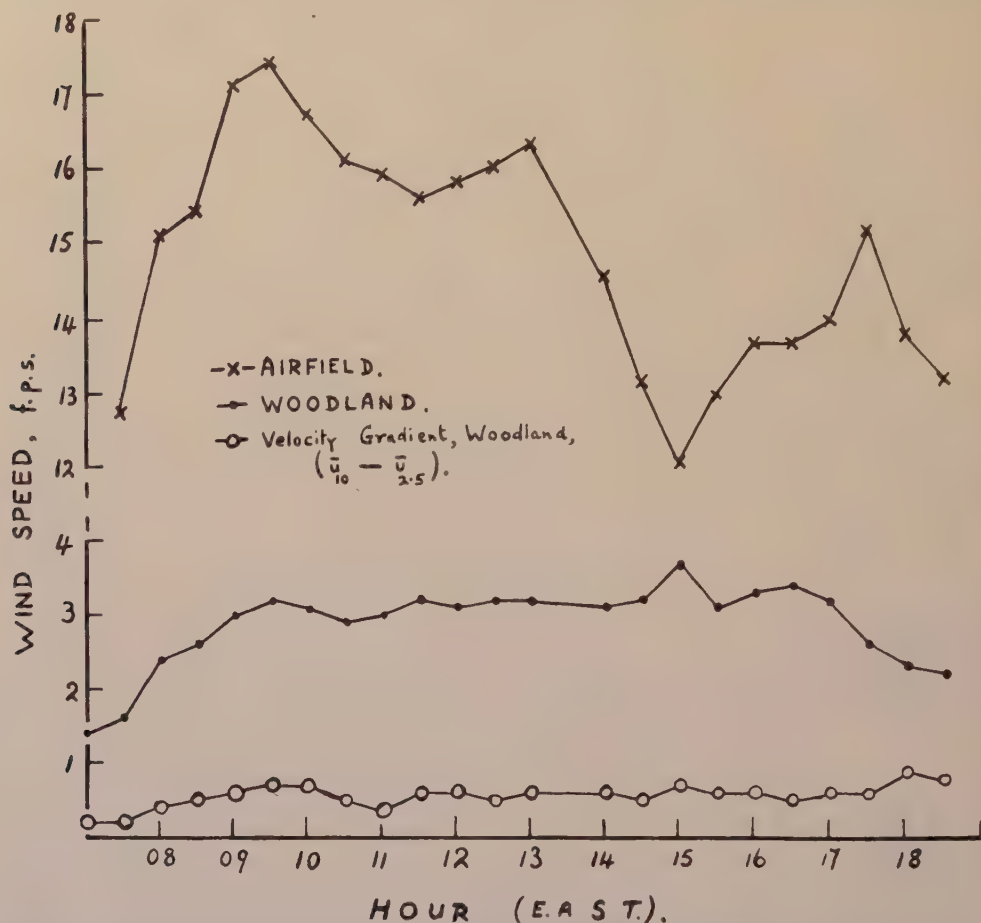


Fig. 2.—Mean half-hourly wind velocity at 10 m. in woodland and open for July 1951. Mean wind velocity gradient, expressed as the difference of wind at 10 and 2.5 m., is also shown.

to the adiabatic value. The mean diurnal variation of temperature difference ($T_{10} - T_{2.5}$) for the month of July, in comparison with corresponding values for the airfield are plotted in fig. 3. There is a most definite absence of the strong inversions found by Hales during daylight, undoubtedly a reflection of the lighter canopy. In the early morning the strongest inversion is found at 08.00 hours ($1\frac{1}{2}$ hours after sunrise), the inversion which builds up in the evening being found not to exist at dawn. The inversion of 08.00 hours probably results through heating of the canopy, but not the ground beneath it, by the low sun. Adiabatic or very slight super-adiabatic lapse rate conditions are soon reached, however, and persist until about 14.00 hours. After this the bush atmosphere is isothermal until 16.00 hours and then becomes increasingly inversionsal. There is no immediately obvious reason why conditions should be super-adiabatic lapse after

08.30 hours in the morning and isothermal in the afternoon until about 16.00 hours, and it is probably better to assume them as in the mean adiabatic throughout the period, variations from this value being extremely slight and

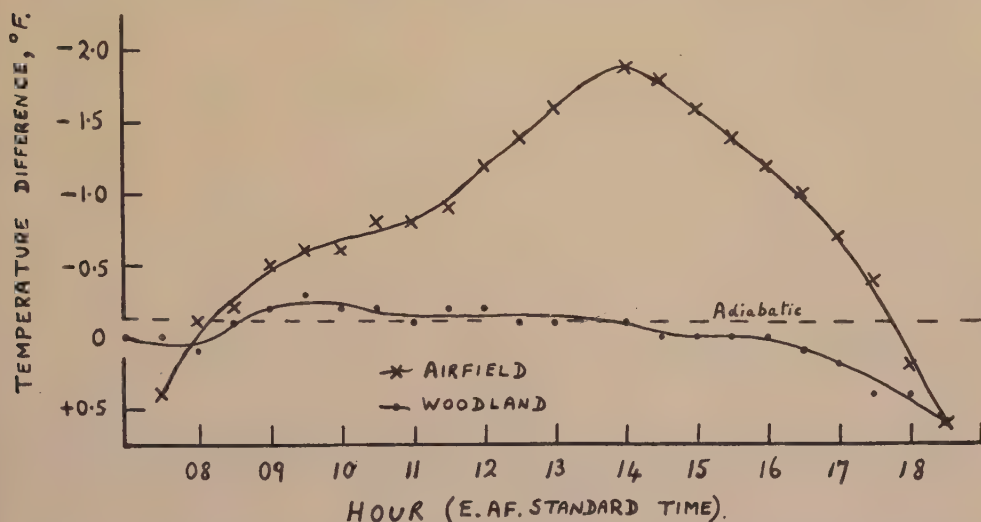


Fig. 3.—Variation of mean temperature difference ($T_{10}-T_{2.5}$) in the woodland and on the airfield, July 1951.

within the observational limits of the psychrometers. The time of commencement of development of the evening inversion in the woodland, soon after 16.00 hours, is rather early. At this time there is probably little direct heating of the ground surface by the lowering sun (sunset 18.30 hours) and therefore much outward radiation, the canopy and upper levels falling in temperature less since the foliage continues to receive heat from the sun and also accepts radiation from the ground beneath. Later, when the canopy begins to radiate, the inversion is presumably destroyed.

Instantaneous measurements of temperature profiles in the bush show that within the lowest 10 m. there is a constant fluctuation of lapse rate about the mean adiabatic position, and throughout the whole depth of the woodland the profiles vary considerably about characteristic forms. Some typical results are shown in fig. 4. All daylight results show a rapid drop in temperature from just above to just beneath the canopy. However, instead of an inversion persisting down to ground level, as Hales' results indicated, temperatures begin to rise again downwards from the canopy. The fairly constant and slow fluctuation of temperature profiles throughout day and night may be traced by considering the profiles shown in fig. 4. Whilst the sun is still low in the morning (sunrise 0620 hours during these experiments) the upper surface of the canopy is first heated, so that a marked temperature fall develops from above to beneath it, as may have been the case in C, D, F and J of fig. 4. Super-adiabatic lapse rate conditions may already occur in the lower part of the bush (fig. 4, G) or they may develop by heating of the ground, as perhaps occurred in examples A, D, F and J of fig. 4. This unstable state is unlikely to persist and develop indefinitely. Assisted by mechanical turbulence, cool air beneath the canopy slumps to the ground, and warmed air near the ground rises convectively towards the foliage and inversion barrier. Thus the upper inversion is weakened as in B (cf. C, $1\frac{1}{2}$ hours earlier), and a new low level inversion develops as in B, C and E. The process is then repetitive throughout the daylight hours, so that the picture of

thermal turbulence in the bush is of slow, continual and necessarily haphazard exchange between upper and lower levels. It is of course this mixing, combined with the mixing due to wind eddying about the trees when the wind speed in the woodland is sufficient, and also combined with heating of both ground and

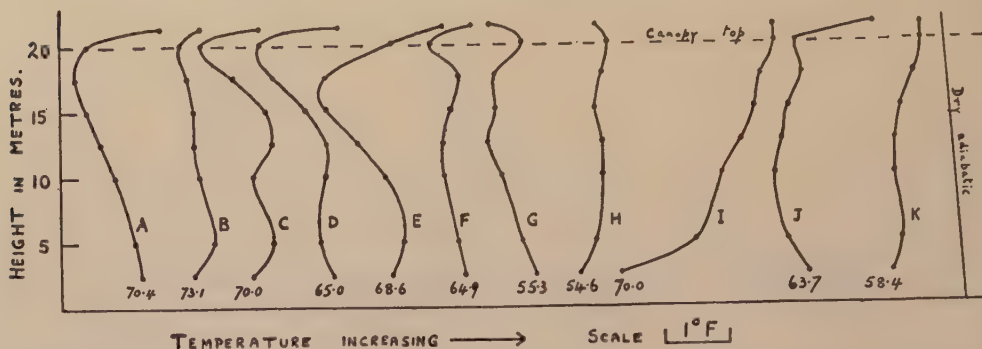


Fig. 4.—Some examples of instantaneous temperature profiles in woodland, September 1951. A 27th 11.22 hrs.: B 2nd 12.20 hrs.: C 2nd 10.50 hrs.: D 5th 09.15 hrs.: E 26th 11.00 hrs.: F 26th 09.25 hrs.: G 28th 06.10 hrs.: H 28th 06.30 hrs.: I 27th 18.08 hrs.: J 25th 10.25 hrs.: K 5th 03.00 hrs.

canopy, which keeps mean temperature profile values at the adiabatic lapse rate during most of the day-time.

The evening development of inversion within woodland has been previously commented upon. An instantaneous evening (18.08 hours) profile is shown as I in fig. 4, and the absence of this inversion in the early mornings, *e.g.*, G and H, 06.10 and 06.30 hours, calls for explanation. In the case of I, temperatures immediately beneath and above the canopy are little different, but the latter will soon begin to fall with radiation from the upper surface of the foliage and cooling of the air in contact with it. (Such an effect, although occurring in the early morning, is probably illustrated in G and H). Radiation also occurs from the lower surface of the foliage towards the ground, but at the same time this foliage absorbs radiation from the ground, and hence temperature diminution beneath the canopy is less than that above. However, as time goes on, and the air above the canopy continues to cool, mixing* inevitably occurs with the warmer air immediately beneath, the temperature of the air within the canopy and in the upper part of the woodland falls, and a large under-canopy inversion such as that of I must inevitably weaken. If above-canopy cooling continues, and is more rapid than ground cooling, a near-adiabatic state is eventually reached and finally an unstable lapse rate may become established beneath the trees, *i.e.*, a super-adiabatic lapse rate developed by cooling aloft rather than by warming at low levels. Gradual slumping towards the ground of pools of cold air from aloft and a compensatory vertical component of motion of air from the surface become possible, thus causing the breakdown of instability and the establishment of weak inversions or near-adiabatic conditions within the woodland. Hales' results indicate a similar effect in that his nocturnal beneath-canopy temperature gradients show considerable fluctuations, although always remaining negative. It will be seen that throughout the night this necessarily haphazard process is repetitive and self-energising owing to differential cooling between canopy and ground, the effect being to cause slow exchange of air throughout the woodland

* This mixing is obviously partly convective and could quite conceivably carry any insecticide near the canopy upwards out of the woodland. An upward transport of insecticide through the canopy into the free air above was in fact observed and is mentioned on p. 624.

with a tendency always towards the creation of a mean adiabatic lapse rate. Examples are G and H in fig. 4. At 06.10 hours on 28th September 1951 (G in fig. 4), there was instability in the lower part of the woodland and an inversion in the upper part; the temperature immediately beneath the canopy was higher than that above. From Table I it can be seen that winds were very light in the free air at 06.20 hours and there was a strong inversion of temperature at the airfield. There was no cloud and sunrise was at 06.20 hours. At 06.30 hours (H in fig. 4) before any solar heating could have been effective, the lapse rate in the lower levels had been replaced by an inversion, the temperature at 2.5 m. having fallen to a value lower than that above the canopy. Whilst this temperature fall could no doubt be attributed to radiational cooling it appears more likely to have been caused by cold air slump since there was no corresponding fall of temperature above the trees, indeed temperatures rose there very slightly. A small temperature increase also occurred in the upper half of the woodland, an advective or convective effect.

It appears that by both day and night thermally initiated air movements occur within this type of woodland. During most of the daylight period they are swamped by frictional turbulence, so that their importance becomes greatest during the night-time in the absence of strong winds.

Bi-directional Wind Vane Results and Visual Observations of Smoke Clouds.

Seventy-three bi-directional wind vane experiments were made in the bush at 10 m. at different times. The resulting traces could not be analysed in the fashion employed by Scrase (1930) and Best (1935), and normally used for observations in the open, since they did not conform to the typical elliptical pattern with marked focal point, but it proved fairly easy to make a classification according to wind speed into the categories given in Table II, regardless of time of day. Examples from each category are given in figs. 5, 6 and 7.

TABLE II.

Classification of bi-directional wind vane traces in woodland at 10 m.

Wind speed.	Character of bi-vane trace.
Below 2 f.p.s.	Laminar flow, generally upward or downward. Slight swinging motion usually apparent. Thirteen observations.
2.5-4 f.p.s.	Irregular traces with no focal point and sluggish and limited movement both vertically and horizontally. Thirty-eight observations.
Above 4 f.p.s.	Irregular traces with no focal point and rapid and very wide vertical and horizontal movements. Twenty-two observations.

With very low wind speeds 12 out of 13 observations showed steady or nearly steady motion in a non-horizontal plane. In fig. 5 (a) the almost regular horizontal trace along the equator of the bi-vane chart indicates horizontal flow with a slight swinging motion. This was the only recorded instance of horizontal flow. Flow in a single plane, again with a swinging motion is shown in fig. 5 (b), but the air movement is downward and inclined at an angle of approximately 22° to the horizontal. A somewhat more unsteady upward flow inclined at approximately 15° - 24° to the horizontal is indicated in fig. 5 (c), and in fig. 5 (d) slightly unsteady upward flow at only a small angle to the horizontal (3° - 6°) is seen to change to a much steeper angle near the end of the period of observation. It is of interest that the four cases of upward motion are all more unsteady than the eight cases of downward movement. Laminar downflow and slightly more

unsteady but still almost laminar upflow thus occur with very light winds, and substantiate ideas of air movements initiated by thermal variations within the woodland. It is unfortunate that no absolute observations of outside wind speed and lapse rate are available for the cases of fig. 5 (a) and (d) when airflow in the

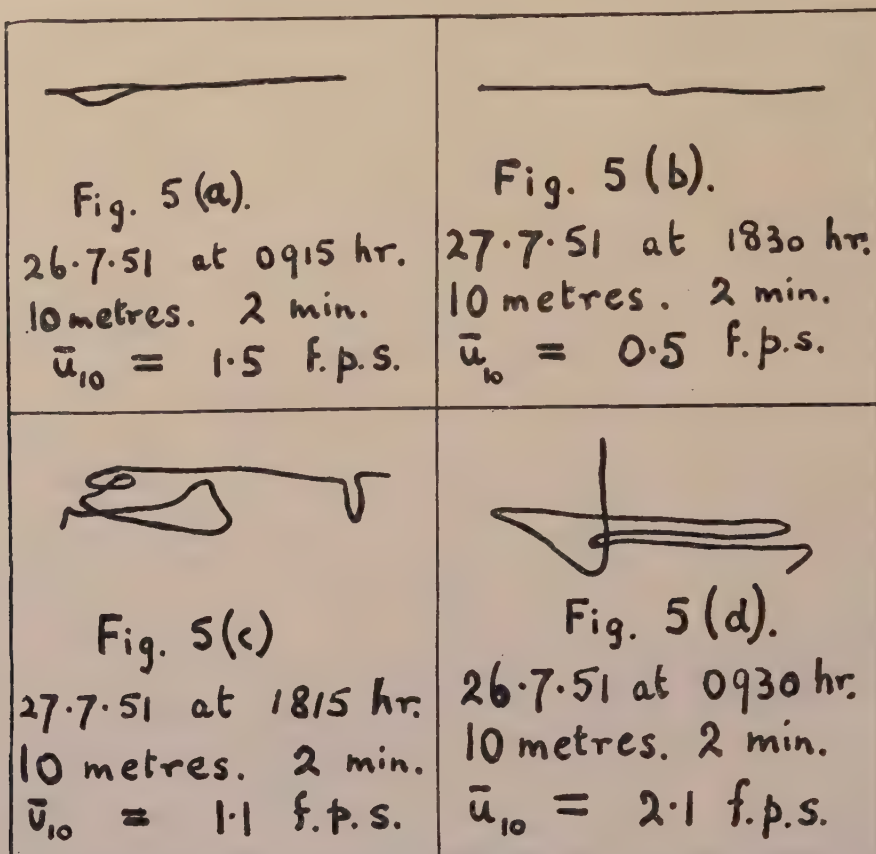


Fig. 5.—Woodland bi-directional wind vane records at 10 m. Laminar flow with wind speed less than 2 f.p.s.

woodland was so nearly laminar. Estimated outside wind speed was 10–15 f.p.s., certainly no more, and since on no occasion during a year's climatological series in the open was there other than a super-adiabatic lapse rate at and after 09.00 hours it is quite safe to assume the same on this occasion, showing that laminar flow in woodland may occur with turbulent conditions outside.

Examples are shown in figs. 6 and 7 of bi-directional wind vane traces when the woodland wind speeds at 10 m. were within the ranges 2.5–4 f.p.s. and above 4 f.p.s., respectively. The effect of frictional eddying about the trees is seen to be considerable at the lower wind speed and very considerable at the higher one. Thus the small thermal motions observable with light outside winds become completely swamped. Since the largest number of winds by day up to 10 m. fell within the range 2.5–4 f.p.s. the overall effect under the particular canopy of the experiment is of slow and rather sluggish eddy movements of the form shown in fig. 6. With lighter canopies or above 10 m., where winds greater than

4 f.p.s. are more frequent, enhanced turbulence is to be expected, and will be similar to that of fig. 7.

The air motion within this woodland was also investigated by direct observation of artificially created clouds of smoke or insecticide. Smoke was made by

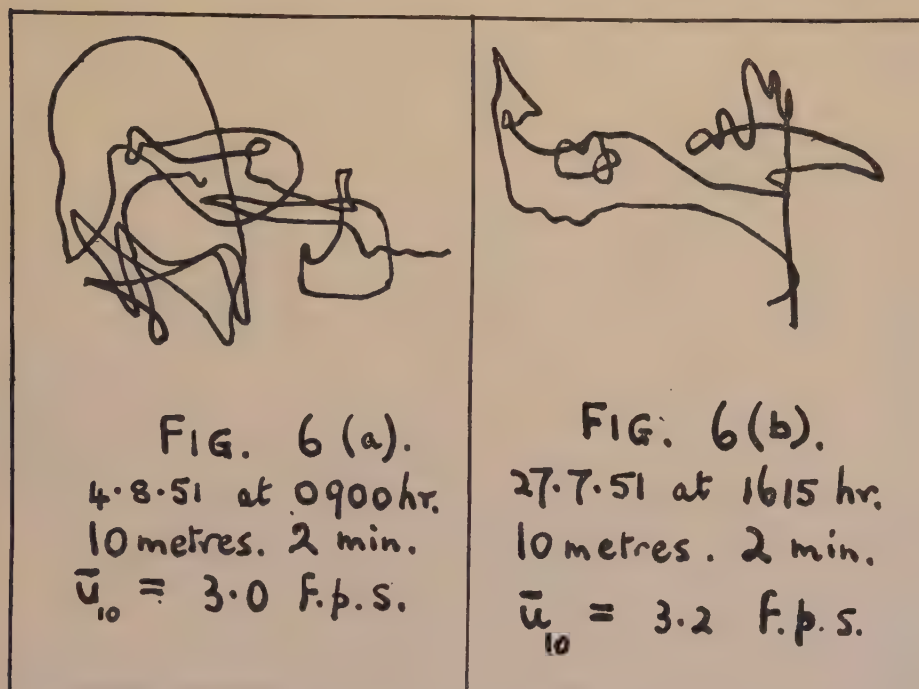


Fig. 6.—Woodland bi-directional wind vane records at 10 m. Turbulent flow within the wind speed range 2.5–4 f.p.s.

suddenly pouring water into a dish of titanium tetrachloride, but more often actual small-droplet insecticide clouds were created from hand generators of the Moskill type, this method being far cleaner and more convenient. Initially, of course, these clouds rise under the thermal convection created by their source heat, but temperature adjustment with the environment takes place well before the cloud has become too diffuse to observe. Since the smoke was created at the meteorological tower, which was the focus of eight paths cut through the woodland for other experimental purposes, it was easy to follow the clouds downwind through the bush.

The persistence of the smoke during the daylight hours, when the lapse rate at the airfield was super-adiabatic, and wind speeds at 10 m. within the woodland were of the order of 2.5–4 f.p.s., was obviously greater at low levels than at high, and its movement slower. Sometimes marked domes of smoke, rising slowly at first, could be seen accelerating upwards and rapidly diffusing as they reached high levels. The movement of the smoke was invariably more sluggish the denser the canopy and in these conditions upward and downward motions were always visible, generally but not always with a slow swirling, or eddy, motion. With increasingly lighter canopies, however, upward motion became more and more prevalent until, eventually, upward movement only could be observed. It was very noticeable that the smoke tended to be attracted towards clearings or very thin canopies—near-horizontal air motion developing to

replace the air lost by ascent due to convection. A somewhat similar effect was suggested by Paton (1948) in connection with air motion within a wheat-field.

During early morning and evening periods of temperature inversion and light winds in the free air, persistence of smoke in the woodland was very marked,

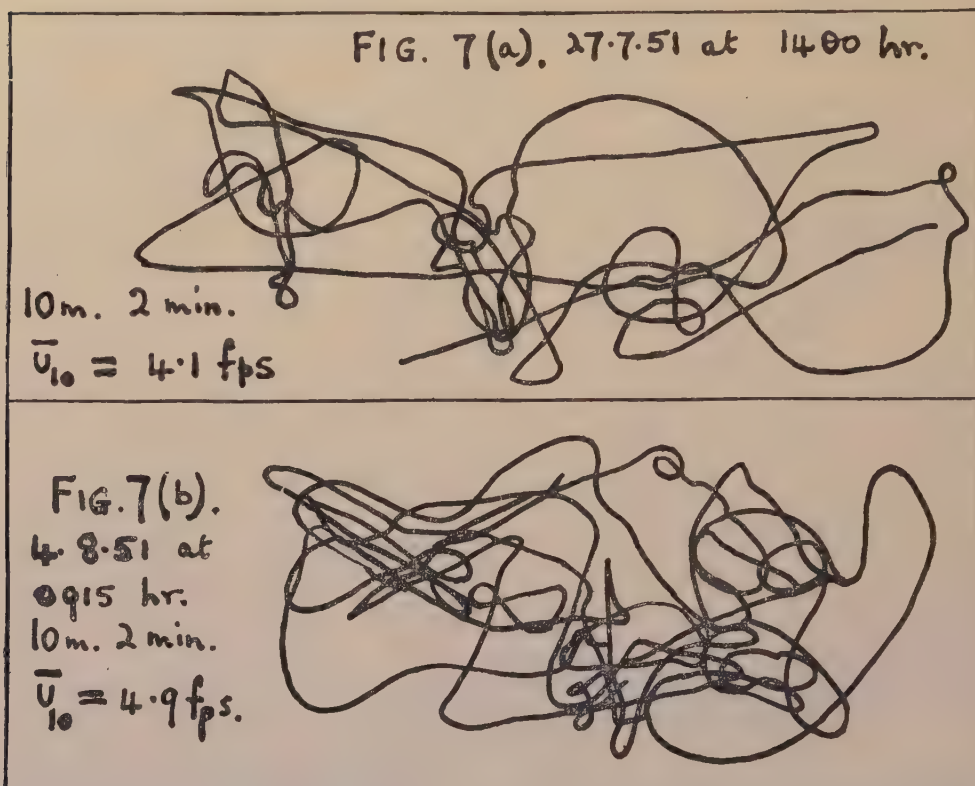


Fig. 7.—Woodland bi-directional wind vane records at 10 m. Very turbulent flow with wind speeds above 4 f.p.s.

but upward and downward movements were still strongly evident; in fact upon one occasion insecticide was observed to rise above the canopy only to sink beneath it again and slowly settle some distance further on. The development of one and occasionally more sheets of smoke was always a dominant feature, air motion often being shown as a widespread lifting or falling of a large extent of the sheet. Sometimes it fell in one part but rose in another. In areas of light canopy or clearings upward movements diminished considerably or were not evident at this time of day, indicating clearly the necessity for a canopy to create the weak upward convective movements. There was no doubt about the longer persistence of the insecticide during these almost calm early morning and evening periods, due almost entirely to the reduced diffusion under conditions of light winds and the absence of strong convection effects. This is obviously the time to choose in order to saturate thoroughly with insecticide any area of woodland, particularly from the air. Ground applications might suffer from patchy dosage in view of the uneven distribution of upward movements due to the presence of clearings or locally thin canopies, but the long airborne persistence of insecticide would reduce even this disadvantage. The lighter the canopy,

however, obviously the less chance of upward movement of ground smoke in these quiet conditions.

Conclusions.

In quiet, non-turbulent, atmospheric conditions slow upward and downward sheet movements of air occur both by day and night beneath moderate and dense woodland canopies, giving widespread dissemination and slow diffusion of introduced insecticide. The extent of dissemination and diffusion obviously also depends upon the settling properties of the droplets used, a high density of very small droplets being the most desirable. Insecticidal applications from the air, relying on sedimentation, are likely to give a high degree of saturation of woodland in non-turbulent atmospheric conditions, providing the canopy is not so very dense that the insecticide is unable to penetrate it. Insecticidal applications from a ground source under moderate canopies and in non-turbulent atmospheric conditions should be just as effective as aerial applications, and under very dense canopies might well be more successful, but with very light canopies there is a danger that insecticide clouds from the ground source would not reach the tops of the trees, unless jet nozzles or a considerably heated source, were used, and then probably only near the point of emission.

During most of the daylight hours the degree of turbulence within the trunk space depends upon the canopy density and the outside wind speed. Although thermally initiated movements beneath moderate and dense canopies exist, increased wind introduces sufficient eddying about the trees to swamp them, the effect being less the denser the canopy. Aerial applications of insecticide during most of the tropical daytime are normally out of the question owing to turbulent diffusion of the insecticide immediately it leaves the aircraft, but it is possible that ground installations would offer success under dense and more especially very dense canopies, where the turbulence tends to be sluggish. Clearings and thin patches, however, would certainly reduce insecticide efficiency by causing rapid diffusion.

An important feature is the variation of wind speed with height within woodland. It is seen from Table I and fig. 2 that wind speeds decrease downwards from the canopy; it follows that the lower the trees the stronger will be the winds beneath the canopy, and hence the greater the turbulence within the trunk space, canopy density being constant.

Summary.

An effort was made to determine the best conditions to achieve maximum saturation and persistence of small droplet insecticide (5-200 microns droplet diameter) within a type of African woodland. The problem is entirely one of atmospheric turbulence within the woodland. No attempt was made, or is likely to be fruitful, to introduce a detailed or mathematical argument. The relevant factors pertaining to atmospheric turbulence were observed and in some cases were directly compared with simultaneous observations made in the open.

The absence of zero wind speeds, considerations of changing lapse rates, bi-directional wind vane records, and visual observations of smoke, all combine to suggest that when conditions of calm or light winds exist in the free air, there is, under moderately dense woodland canopies, an almost continuous and slow transfer of air between the canopy and the ground, these movements tending towards laminar or sheet flows. With stronger winds, eddying about the trees swamps these gentle movements and air motion becomes erratic and turbulent, although such turbulence is less the denser the canopy. During the daytime there is notable horizontal movement of air towards clearings where convection is at a maximum.

Persistence and high saturation of insecticide will be achieved when conditions are quiet outside, and, due to the slow but widespread air movements, good distribution will be attained in these conditions. The lighter the woodland canopy, however, the smaller these air movements become, since they result from differential heating or cooling between canopy and ground, and the more valuable become aircraft applications relying upon the sedimentation of insecticide. Very dense canopies could probably be satisfactorily treated from the ground even during the highest lapse period of the tropical day.

Acknowledgements.

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References.

- BEST, A. C. (1935). *Geophys. Mem.*, Lond., no. 65.
DEACON, E. L. (1949). *Quart. J. R. met. Soc.*, **75**, p. 89.
GEIGER, R. (1950). *The climate near the ground.*—Cambridge, Mass., Harvard Univ. Pr.
HALES, W. B. (1949). *Bull. Amer. met. Soc.*, **30**, p. 124.
PATON, J. (1948). *Weather*, **3**, p. 22.
SCRASE, F. J. (1930). *Geophys. Mem.*, Lond., no. 52.
SUTTON, O. G. (1949). *Atmospheric turbulence.*—London, Methuen.
TAYLOR, G. I. (1927). *Quart. J. R. met. Soc.*, **53**, p. 201.
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AIRCRAFT APPLICATIONS OF INSECTICIDES IN EAST AFRICA.

IV.—THE APPLICATION OF COARSE AEROSOLS IN SAVANNAH WOODLAND CONTAINING THE TSETSE FLIES *GLOSSINA MORSITANS* AND *G. SWYNNERTONI*.

By K. S. HOCKING, H. C. M. PARR, D. YEO, and D. ANSTEY.

Colonial Insecticide Research Unit, Arusha, Tanganyika.

Y. E.

In South Africa a considerable effort has been made to eradicate tsetse flies by applying insecticides from aircraft (see, for example, Fiedler, 1950). The experiments showed that coarse aerosols were more effective than sprays. A similar conclusion was reached from experiments carried out at Entebbe, Uganda (Hocking & Yeo, 1953); sprays and aerosols were applied to densely forested islands in Lake Victoria, and significant kills of *Glossina palpalis* (R.-D.) were obtained only on the islands treated with aerosols. A further experiment (Hocking & others, 1953) showed that, in savannah woodland, a coarse spray of an oil solution containing DDT produced relatively low kills of *G. swynnertoni* Aust.

It was consequently decided to investigate the effect of coarse aerosols upon populations of *G. morsitans* Westw. and *G. swynnertoni*, two very important species of tsetse flies in East Africa. Two blocks of typical savannah woodland, each of a few square miles, were treated, one with DDT and the other with BHC. The applications were made during the latter part of the long dry season, from the end of July to the beginning of November, 1949. During this period most of the trees were completely leafless, the ground was hard and dry and it was possible to move about in the area with ease.

Description of the Area.

General.

The experimental area was near Kikore village (Lat. 04°21'S., Long. 33°19'E.) in the Central Province of Tanganyika. It was approximately 4,200 ft. above sea level. Originally the fly belt in the area consisted of a strip of woodland along the eastern base of the Masai escarpment; it extended eastwards for a few miles, and on its eastern side was bounded by the "Great Mbuga", part of the Masai steppe. In recent years extensive clearings have been made, and it was relatively easy to obtain blocks of woodland that were quite well suited for our experiments. An approximate map of the area is given in fig. 1, which shows the two woodland areas which were treated with the insecticidal aerosols, the woodland area in which control catches of flies were made, and the general topographical features of the district.

Flora.

In fig. 2 a detailed map is given of the North Block, which was treated with DDT. Vegetation communities are shown, and the map includes the paths upon which catches of flies were made, roads, and other relevant details.

Most of the woodland consisted of a mixed stand of *Combretum-Dalbergia* spp., with the secondary trees *Commiphora* spp., *Lannea humilis*, *Vitex* sp., and *Euphorbia bilocularis*. On the western side of the block there were two important areas of woodland commonly called "miombo", consisting mainly of *Isoberlinia*

globiflora but including some *Brachystegia microphylla*. Along the northern edge of the block there was a belt of the tall acacia trees *Acacia spirocarpa*, *Acacia xanthophloea*, and *Acacia usambarensis*. Scattered trees of *Acacia spirocarpa* and *Acacia usambarensis* were found over most of the block, and other secondary

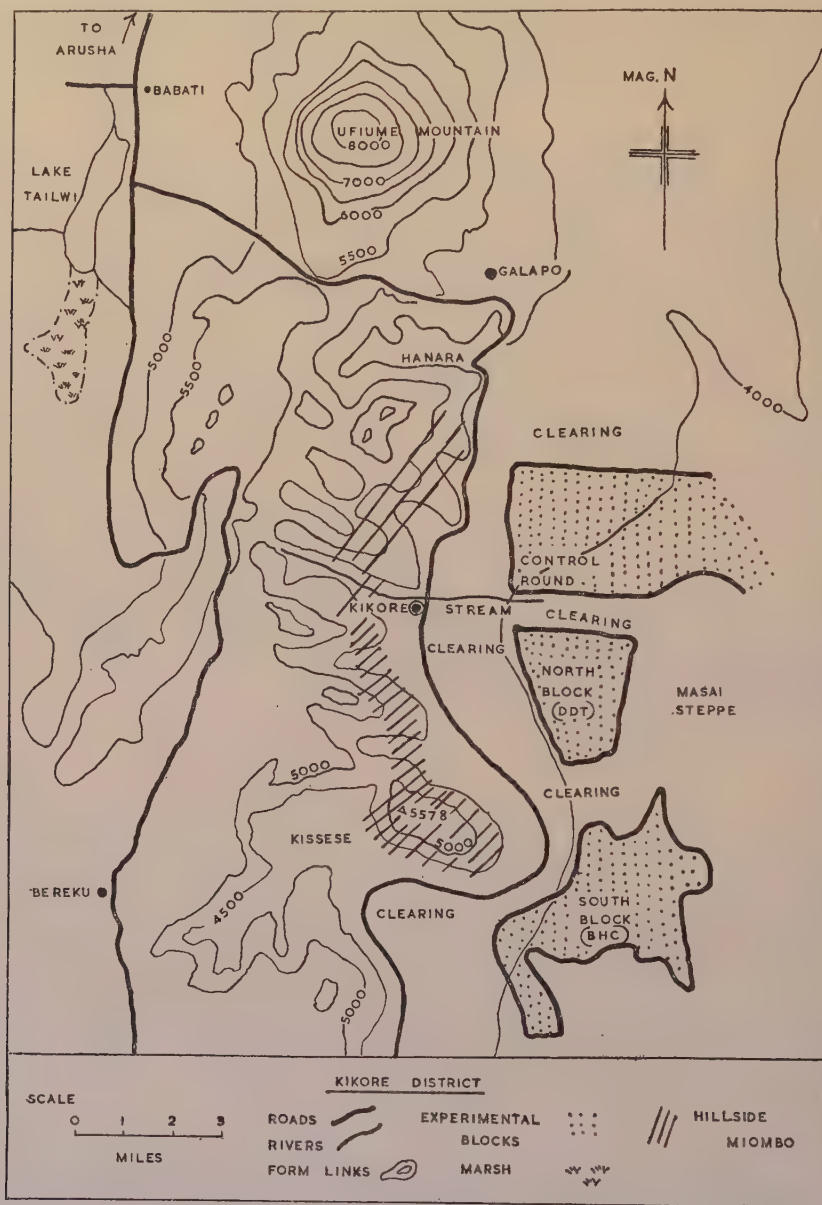


Fig. 1.—An approximate map of the Kikore area, showing the two experimental blocks, other fly-infested woodland, and general topographical features.

trees were gall acacia (*Acacia drepanolobium* and *Acacia formicarum*), *Kigelia aethiopica*, *Crossopteryx febrifuga*, and *Gardenia thunbergia*.

The block treated with BHC, and known for convenience as the South Block, is shown in fig. 3. Its eastern part was covered with "nyika" thicket, mainly of the *Acacia* spp. and *Commiphora* spp. The dominant trees were *Acacia kirkii*, *Acacia subulata*, *Commiphora* spp., and *Fagara merkeri*; other trees were *Acacia*

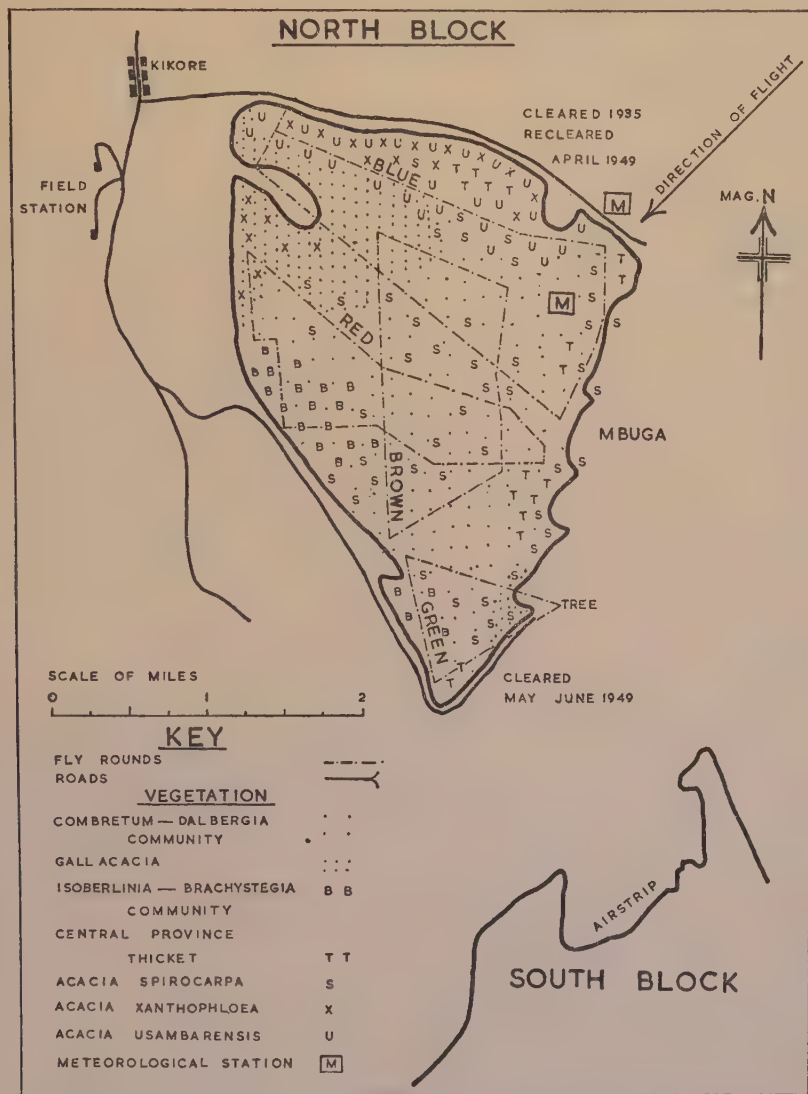


Fig. 2.—A detailed map of the North Block, the woodland treated with DDT.

stulmannii, *Acacia fischeri*, *Maerua crassifolia* and *Acacia mellifera*. There were also scattered shrubs of *Grewia bicolor*, *Cassipourea mollis* and *Capparis* sp. Much of the rest of the woodland was of the "Central Province Thicket" type and was very similar to that described as "dry ravine thickets" by Burt (1942), and there were many relatively open areas containing *Acacia spirocarpa*, *Commiphora* sp., *Dalbergia* sp., *Markhamia obtusifolia*, and gall acacia.

Distribution of tsetse flies within the two experimental blocks.

The North Block was principally the habitat of *G. morsitans*. Breeding was most concentrated in the stands of "miombo" (B, in fig. 2); in a pupal survey carried out just as the insecticidal applications started 30 per cent. of the pupae were found there. The population of *G. swynnertoni* was smaller than that of *G. morsitans*, and was concentrated in the eastern fringe of the block.

Most of the South Block was typically the habitat of *G. swynnertoni*, which was more numerous than *G. morsitans*. In a pupal survey, most of the pupae of *G. swynnertoni* were found in the eastern part of the block whilst the population of *G. morsitans* was mainly confined to the north-western part.

The isolation of the blocks.

The isolation of the two treated areas was not entirely satisfactory, but little could have been done to improve it without great expense. Large areas would have had to be cleared of trees, and this in itself would have been expensive. In addition, however, it would have delayed by several months the start of the experiment, and during this time the costly hire of the aircraft would still have had to be met even though there would have been no flying.

Large numbers of *G. morsitans* lived in the "miombo" on the slopes of the escarpment, which in some places was only a mile or less from the treated areas. The control catches were made in woodland where there was a high population density of *G. morsitans* and some *G. swynnertoni*, and this woodland was only 1,000 yds. from the North Block. Bordering the eastern edge of the South Block were patches of gall acacia and *Acacia seyal*, and there was also a small patch of "nyika" (see p. 629) thicket which contained a very small population of *G. swynnertoni*. There was, therefore, a definite possibility that flies would move into the treated areas from nearby fly-infested areas of woodland.

The Control of Dosage and Field Methods.

Each block was treated with a 10 per cent. w/v solution of the insecticides; technical DDT (80 per cent. p,p'isomer) was used for the North Block and crude BHC (12 per cent. γ isomer) for the South Block. The solvent consisted of 1 part Shell Furnace Oil to 4 parts Shell Power Kerosene. It was heated to 106°F. during mixing so that the insecticide dissolved readily. The strength of the DDT solution was always very nearly the required value, but not all the BHC remained in solution and a large amount of sludge was obtained. Much of this was removed by filtering, and chemical analyses showed that the final solution contained only 7 per cent. w/v of total BHC. The relative solubilities of the various isomers are such, however, that the amount of γ isomer must have been very close to the required value of 1.2 per cent. w/v.

The factors governing the frequency and the number of applications have been discussed elsewhere (Hocking & Yeo, 1953). Eight applications were made, at intervals of approximately 2 weeks, in order to cover a period somewhat longer than two pupal periods. Each application was carried out at a nominal dosage of solution of 0.25 gallons per acre, *i.e.*, either 0.20 lb. per acre of the p,p'isomer of DDT or 0.03 lb. per acre of the γ isomer of BHC. Previous (unpublished) work had suggested that at these proportionate dosages the two insecticides would be equally effective.

The aerosols were produced by allowing the insecticidal solution to flow into modified exhaust systems fitted to the engines of the aircraft. The apparatus has been described elsewhere (Hocking & Yeo, 1953). The aerosol had droplets varying in diameter from 4 microns to 250 microns approximately, and had a mass median diameter of approximately 90 microns. The solution was emitted under gravity, and the emission rate fell as the storage tanks emptied. At the

beginning of a normal sortie of which, owing to meteorological conditions, it was usually only possible to make one per day, and which consisted of a number of parallel swathes, the emission rate was approximately 7 gallons per minute from each exhaust system, and it fell to just over 2 gallons per minute when the tanks were nearly empty. There was no means whereby this wide variation could have been eliminated with the apparatus that was used, and a constant nominal dosage could have been obtained only by complicated variations of the swathe width.

Instead of varying the swathe width the following system was adopted. Successive sorties upon adjacent areas of woodland were overlapped so that 25 per cent. of the total solution was used to re-treat areas which had received low dosages towards the end of the previous sortie. The runs in the overlap were 82.5 yds. apart, but in the main part of each sortie they were 55 yds. apart, giving a swathe of that width. The distribution of nominal dosages is illustrated in fig. 4, where for convenience a constant length of run of two miles has been

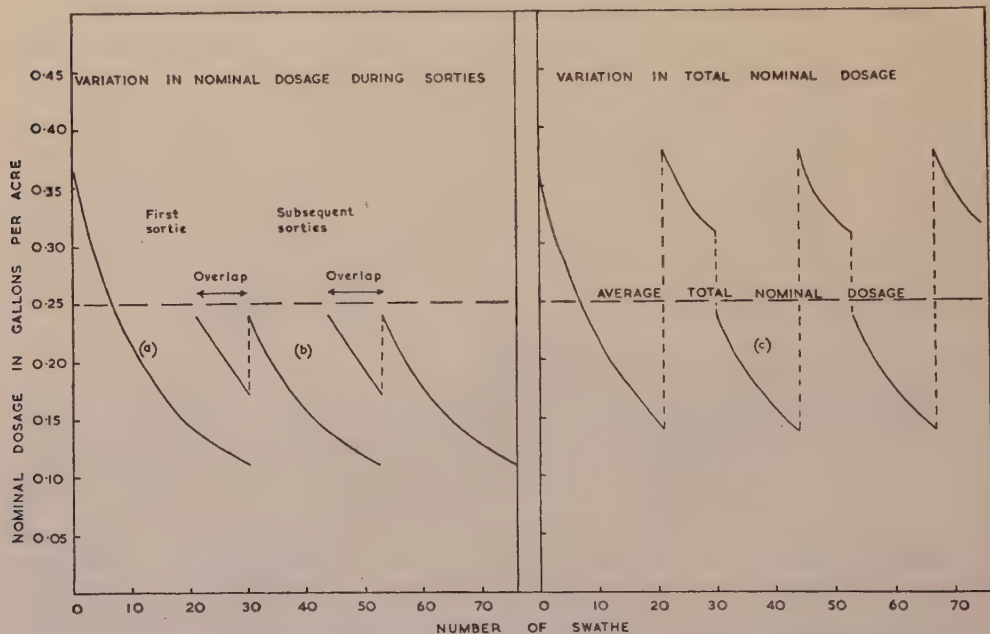


Fig. 4.—The distribution of nominal dosages: (a), dosage distribution for the first sortie on a block; (b), dosage distribution for subsequent sorties; (c), variation in total dosage across the swathes. A constant length of run of 2 miles has been considered.

considered. The distribution of dosage for the first sortie on a block is shown by graph (a); graph (b) shows the dosages during subsequent sorties, while graph (c) shows how the total dosage varied across the swathes. The graphs are idealised forms of the dosage distribution; they are the basis upon which the applications were made, but the actual dosages varied somewhat from those shown.

The overlap served three purposes.

(a) Areas treated towards the end of a sortie received dosages of only 45 per cent. of the average nominal dosage, and were usually treated during relatively poor meteorological conditions since the favourable conditions which prevail about dawn are known to deteriorate very rapidly. These areas were again treated in the overlap which, being performed at the start of the next daily

sortie, was usually during good meteorological conditions, and in this way no area received a dosage less than 56 per cent. of the average nominal dosage.

(b) Between daily sorties upon adjacent areas of woodland, flies were free to move from untreated areas to areas that had been already treated. Since the effectiveness of the aerosol depended on contact with the flies by airborne droplets, such flies would not have been killed unless an overlap had been used.

(c) Between successive sorties there were, inevitably, small variations in the tracking of the aircraft. The overlap eliminated the possibility that areas would not be treated because of these small tracking errors. In the North Block it was possible to have an overlap 450–500 yds. wide, whereas because of the greater length of the runs in the South Block the discharge of 25 per cent. of the total solution was effected in a smaller number of swathes and the overlap there was usually only 250–300 yds. wide.

The beginning of each run was indicated to the pilot by a marker which consisted of a strip of white cloth stretched between two poles. The pilot flew over the marker, and continued to make the run on a compass course. The required compass course was obtained during a preliminary run over two permanent markers placed in open grassland along the desired direction. During the runs of each sortie an observer in the aircraft measured the drift due to the wind, so that a check could be kept upon the accuracy of the tracking.

In the South Block the runs were often four miles long, and assessments showed that errors in tracking were sometimes causing large variations in dosage in the areas farthest from the marker. For the later applications a second marker line was therefore used. This passed through the block, and was about two miles from the main marker. Advantage was taken of open areas, and for most runs the marker on this second line was clearly visible to the pilots, and considerably improved the accuracy of tracking.

Two Anson aircraft were based at Arusha, 90 miles from Kikore. This was the nearest suitable aerodrome, although an emergency landing strip was constructed near the South Block, and this was used occasionally for evening sorties so that the cycle of applications could be maintained. Radio and visual signals were used to communicate between the aircraft and the various observers on the ground. The radio was also very useful to contact the aircraft base at Arusha, for example when the local weather conditions at Kikore were unsuitable.

Aerosol Behaviour and Meteorological Conditions.

The behaviour of coarse aerosols is very complex and is greatly affected by the meteorological conditions and the type and density of the vegetation cover. The degree of atmospheric turbulence affects the concentrations of aerosol to be found near the ground, and the vegetation cover affects the amount of insecticide that reaches the resting places of the tsetse flies. The best periods for applications of aerosols are usually near dawn and dusk; at these times atmospheric turbulence tends to be slight, and conditions approach the ideal state of high temperature inversion and low wind speed.

The extent of the problem of variations in aerosol behaviour was not fully realised during these experiments, and it was not until later than more informative data were collected. Some general comments are possible, however. The aircraft took 45 minutes to reach the experimental area, and for the morning sorties this meant that the best period for applying the aerosols was wasted. Evening sorties were often unsatisfactory because of high winds and so were only occasionally made. For most of the time the temperature gradients were of the dry adiabatic lapse rate, or slight super-adiabatic lapse rate, form, and wind speeds were rarely more than 6 ft./sec. From later work it seems likely that about 30 per cent. of the sorties were carried out during poor conditions; in particular, important breeding areas in the south-eastern and north-eastern

tongues of the South Block probably never received a treatment during good meteorological conditions. At the beginning of the series of applications heavy layers of low strato-cumulus cloud delayed the onset of extreme turbulence; towards the end of the applications clear mornings with bright sun often occurred, and it is probable that the later applications were less effective.

It should be remembered that there was little choice of time for carrying out the sorties. The two areas totalled 15 sq. miles, and had to be treated each fortnight. Usually each application took 14 sorties with two aircraft, and occupied 11 or 12 days, *i.e.*, at an average of little more than one sortie per aircraft per day. This left little time to carry out the necessary maintenance on the aircraft, and to maintain the sequence of applications it was inevitable that certain areas were poorly treated.

Quite early in the experiment it was evident that some system was required for deciding whether or not atmospheric conditions were too turbulent for effective application of the coarse aerosols. The smaller droplets in the aerosol cloud were clearly visible as a white cloud for several seconds after emission. It was observed that during stable conditions of inversions with low wind speeds this "cloud" sank rapidly into the trees, and none of it was then visible above the top of the canopy. As conditions deteriorated, so the rate at which the cloud settled into the trees decreased, and under unstable conditions the aerosol cloud billowed above the aircraft. Accordingly when billowing became pronounced the sorties were abandoned.

The filtering and screening effects of vegetation cover will be discussed elsewhere. It was found that the droplets with diameters greater than 80 microns did not reach the leeward sides of obstacles, nor did they penetrate into thickets. These larger droplets must therefore have been relatively ineffective in killing flies. Since over 50 per cent. of the insecticides was contained in these larger droplets, the drop spectrum was not ideal.

Entomological Observations.

Four fly paths passing through the main vegetation communities were laid out in each experimental block. Catches were made in the usual way by two African assistants who were often accompanied by a European supervisor. All paths were traversed weekly. During the applications an attempt was made to carry out catches immediately after an application in an area, and also during the intervening week. Since, however, no fly path coincided with areas treated during one sortie, catches were usually made 1-3 days, and 7-10 days, after each application.

Before the experiment started the populations of non-teneral male flies were estimated, using a marking technique adapted from the original work of Jackson (1948). The results are given in Table I; only the estimates for *G. morsitans*

TABLE I.

Estimates of the populations of non-teneral male flies in the two experimental blocks.

Place	Species	Population
North Block	<i>G. morsitans</i>	8,000
North Block	<i>G. swynnertoni</i>	(350)
South Block	<i>G. morsitans</i>	—
South Block	<i>G. swynnertoni</i>	5,000

The above figures apply to June, 1949.

in the North Block, and of *G. swynnertoni* in the South Block, are reliable. The female populations were probably approximately double the male populations.

The fly catches are given in Tables II, III and IV, where apparent densities, *i.e.*, the number of non-teneral male flies caught per 10,000 yds. of fly path (see

TABLE II.

Summary of the catches of flies in the North Block, the area treated with DDT.

Date	<i>G. morsitans</i>		<i>G. swynnertoni</i>	
Week ending	Apparent density	All flies	Apparent density	All flies
4.vi.49	202	734	24	104
11.vi	161	559	29	126
18.vi	148	476	23	129
25.vi	142	473	38	129
2.vii	120	430	21	64
9.vii	138	463	33	118
16.vii	163	537	35	119
23.vii	147	523	46	154
FIRST APPLICATION				
30.vii.49	10	38	3.3	11
SECOND APPLICATION				
6.viii.49	1.7	8	0.7	3
13.viii	2.3	9	0.7	2
THIRD APPLICATION				
20.viii.49	0.3	2	0.7	2
27.viii	0.7	5	0.3	2
FOURTH APPLICATION				
3.ix.49	0	1	0	3
10.ix	0.3	7	0	0
FIFTH APPLICATION				
17.ix.49	0	0	0.7	2
24.ix	0.3	5	0.3	3
SIXTH APPLICATION				
1.x.49	0.3	1	0	0
8.x	0	2	0.3	1
SEVENTH APPLICATION				
15.x.49	0	2	0	0
22.x	0.3	1	0	0
EIGHTH APPLICATION				
29.x.49	0	0	0.3	1
5.xi	0	0	0	0
12.xi	0	1	0.3	1
19.xi	0	0	0	0
26.xi	0	2	0	0
xii.49	0	0.2	0.2	0.4
Jan. 1950	0.2	1.0	0.2	1.8
Feb.	0.2	2.8	0.8	5.0
March	0.8	6.0	1.5	10.0
April	1.3	7.0	1.3	9.0
May	1.2	10.0	2.8	15.0
June	2.4	12	3.5	16
July	0.5	2	1.7	7
Aug.	5.2	18	4.7	24
Sept.	5.7	21	3.3	14
Oct.	5.8	18	3.7	18
Nov.	6.2	24	2.3	13
Dec.	5.1	36	3.4	23
Jan. 1951	5.1	32	1.8	14

TABLE III.

Summary of the catches of flies in the South Block, the area treated with BHC.

Date Week ending	<i>G. morsitans</i>		<i>G. swynnertoni</i>	
	Apparent density	All flies	Apparent density	All flies
11.vi.49	17	50	77	223
18.vi	13	49	116	346
25.vi	5.2	17	76	226
2.vii	11	34	95	262
9.vii	11	31	98	300
16.vii	8.5	23	109	310
23.vii	19	62	92	272
FIRST APPLICATION				
30.vii.49	0	0	4.1	14
6.viii	0.4	1	2.6	18
SECOND APPLICATION				
13.viii.49	0	0	0.4	1
20.viii.49	0.4	1	2.6	14
THIRD APPLICATION				
27.viii.49	0	0	0	0
3.ix	0	1	0.4	9
FOURTH APPLICATION				
10.ix.49	0	1	3.7	25
17.ix	2.2	7	16.3	62
FIFTH APPLICATION				
24.ix.49	0	2	1.5	4
1.x	1.1	4	3.7	14
SIXTH APPLICATION				
8.x.49	0	0	—	—
15.x	0	0	2.6	10
SEVENTH APPLICATION				
22.x.49	0.4	1	0.4	1
29.x	0.4	2	1.8	5
EIGHTH APPLICATION				
5.xi.49	0.7	2	0.4	3
12.xi	0	1	1.1	7
19.xi	0.7	3	3.0	15
26.xi	0	1	2.5	12
xii.49	1.3	4	11.0	35
Jan. 1950	0.8	3	13	44
Feb.	0.8	4.5	14	53
March	0.9	3.5	13	46
April	0.7	4.0	15	61
May	1.1	5.0	19	70
June	0	3.0	10	42
July	0	1.5	9	36
Aug.	0.6	2.5	7.4	28
Sept.	0.2	1.0	8.0	26
Oct.	0.7	4.0	5.0	18
Nov.	0.7	4.0	2.6	13
Dec.	1.1	5.0	7.8	26

Date Week ending	<i>G. morsitans</i>		<i>G. swynnertoni</i>	
	Apparent density	All flies	Apparent density	All flies
Jan. 1951	1.8	6.0	7.8	33
Feb.	0.7	2.0	7.8	26
March	1.1	4.0	16.6	51
April	0.7	3.0	8.1	28
May	0.7	5.0	6.7	23
June	2.6	9.0	7.8	25
July	3.0	8.0	8.1	27
Aug.	1.1	3.0	3.7	12
Sept.	0.4	1.0	1.1	7
Oct.	1.0	4.0	2.2	8
Nov.	0.4	1.0	2.2	6
Dec.	0.7	2.0	1.4	5

TABLE IV.

Catches of *G. morsitans* upon the control path.

Month	Apparent density	All flies
July 1949	225	82
Aug.	280	105
Sept.	190	72
Oct.	220	82
Nov.	197	71
Dec.	180	65
Jan. 1950	321	112
Feb.	295	114
March	318	115
April	315	113
May	266	100
June	202	84
July	234	97
Aug.	296	108
Sept.	135	51

Area treated with insecticides from October 1950.

Swynnerton, 1936), and the weekly catches of all flies are recorded. To reduce the lengths of the Tables, all the catches in the area used as a control, and the later catches in the two treated areas, have been condensed so that average values are given of the apparent densities, and of the weekly catches of all flies, for each month.

The catches shown in Table IV suggest that there would have been a normal seasonal variation in the populations of the two treated blocks if the applications had not been carried out; the populations would have decreased slightly over the period of the applications, and by January, 1950, would have risen to slightly above their pretreatment levels.

The final reductions in populations may be summarised in the following manner.

North Block.

G. morsitans. No non-teneral male flies were caught during the eight weeks immediately following the last application. Flies were caught in subsequent

months; the apparent density, particularly of *G. morsitans*, had risen considerably by August, 1950, but from then until January, 1951, there was no significant change for either species of fly.

G. swynnertoni. During the eight weeks after the last application the catches of all flies, and the apparent density, were only 0.4 per cent. of their pretreatment values. The subsequent variations in the population were similar to those of the population of *G. morsitans*.

South Block.

During January and February 1950, the apparent densities of *G. morsitans* and *G. swynnertoni* were 9 per cent. and 12 per cent., respectively, of their pretreatment values; the corresponding figures for catches of all flies are 12 per cent. and 14 per cent. There was no significant rise in populations during the following two years; indeed, if any change occurred the populations decreased.

Discussion.

The catches show that the greatest reductions in fly populations occurred in the North Block, the area treated with DDT, and they also suggest that for a short period the population of *G. morsitans* there had been eliminated. The greater reductions in the North Block might have been due to a variety of reasons, and the differences between the blocks and their treatments may be summarised as follows:—

1. Different insecticides.
2. The type and density of vegetation cover.
3. The relative importance of the infiltration of flies.
4. The distributions of the fly populations.
5. Variations in meteorological conditions.
6. The shapes of the blocks.
7. The widths of the overlaps.

Previous (unpublished) laboratory work had shown that the γ isomer of BHC was approximately 6 times as effective as the p,p' isomer of DDT in killing tsetse flies. Attempts were made to measure whether appreciable decomposition occurred of the insecticides when they were in contact with the hot exhaust gases from the aircraft engines. The results varied considerably, but they suggested that approximately 10–20 per cent. of both DDT and BHC was decomposed. Furthermore, the catches after the first three applications suggested that kills in the South Block, which was treated with BHC, were somewhat higher than kills in the North Block. There is therefore no evidence that the BHC was less effective than the DDT, except that the final reduction in fly populations was less.

The two blocks had different main types of vegetation cover. Apart from thickets, the vegetation was more open in the South Block, and was probably less effective in shielding the flies from the insecticide. Again, since the first three applications in the South Block were more effective than in the North Block, it is improbable that differences in vegetation type and density, which remained relatively constant during the period of treatment, were the principal cause of the differences between the final reductions in the two blocks.

The distribution of flies within a woodland would be important if areas of high population density coincided with areas of poor applications. The North Block was very much more compact than the South Block, and its "miombo" woodland (B, fig. 2), which had a high population density, was treated under good meteorological conditions on all but one occasion. The South Block, on the other hand, was irregular in shape, and less easy to treat, and important breeding areas for *G. swynnertoni* were rarely, if ever, treated during stable atmospheric

conditions. Visual observation of the aerosol cloud suggested that, by chance, conditions were often less suitable during applications upon the South Block; certainly more sorties were abandoned there because of adverse weather.

A smaller overlap was used in the South Block: a width of 250–300 yds. compared with 450–500 yds. in the North Block. As a result more flies might have escaped the treatments by moving between sorties (see p. 633). There are no suitable movement data upon which an estimate of the extent of this type of escape might be made. Work by Yeo (unpublished), which was based on some figures kindly supplied by Dr. C. H. N. Jackson, suggested that for applications similar to those described in this paper, flies that escape destruction by movement might form a considerable proportion of the total number of survivors unless the overlap extended to a depth of approximately 500 yds.

Although it is not conclusively shown that the two insecticides were equally effective at the dosages used, the evidence suggests that the reduced effectiveness of the applications in the South Block was the result of a combination of circumstances, of less effective applications upon the important fly concentrations, more adverse meteorological conditions during the actual sorties, difficulties in treating the area, and an inadequate overlap.

The subsequent fluctuations in the populations of flies are interesting. It is often held that an insect population recovers quite rapidly from insecticidal treatments unless it is completely wiped out, and this is certainly true in most cases. In the South Block, however, the populations of tsetse flies seemed to decrease rather than increase during the two years following the treatments; this is evidence that infiltration of flies into this area was slight, and could not therefore have been the cause of the reduced kills there. In the North Block the populations rose considerably during the months following the applications, but it was proved, by marking flies, that during this period flies were moving into the treated area from the woodland situated to the north. During July and August, 1950, the clearings around the North Block were extended and improved, and late in September, 1950, insecticidal applications were started in the nearby woodland to the north, and the fly populations there reduced to a very low level; from August, 1950, to January, 1951, the populations in the North Block showed no great tendency to rise, presumably because immigration had then ceased to be appreciable. The behaviour of the fly populations subsequent to the applications, particularly in the South Block, thus suggests that a large reduction in a population of tsetse flies, produced by insecticidal applications and involving no change in the habitat, might well be permanent if there was no appreciable movement of flies into the area.

The experiments indicated the need for a detailed study of the effect upon the behaviour of coarse aerosols of variations in meteorological conditions and vegetation cover. Useful data have since been collected, and these are to be published elsewhere. They show that in tropical Africa only a very limited period of daylight is normally available for the successful applications of aerosols, generally for the first hour after dawn and occasionally for a shorter period before dusk. For aircraft applications it is therefore most important to have an aerodrome close to the experimental area, so that applications may be made at the most suitable times as flying at night is forbidden over most of Central Africa.

The drop spectrum was not ideal, since much of the insecticide was contained in droplets that were too large for an effective penetration of vegetation. A general reduction in droplet size would improve the effectiveness of the aerosol; a reduction in the *range* of droplet sizes would also be an advantage, but it is difficult to achieve this and have an apparatus that is suitable for field work. An obvious improvement would be a constant emission rate regardless of the amount of liquid in the storage tanks. It would also be preferable to have an

apparatus which would produce the aerosol without any decomposition of the insecticide.

The reductions in the fly populations were very high, and raised hopes that in isolated areas flies could be eradicated if due attention were paid to all the factors which contributed to the incomplete success of the present experiments.

Summary.

Attempts have been made to eradicate the tsetse flies *G. morsitans* and *G. swynnertoni* from two blocks of savannah woodland situated in the Central Province of Tanganyika.

The insecticides were applied from aircraft. Coarse aerosols were used, with mass median diameters of approximately 90 microns; droplet diameters varied from 4 microns to 250 microns approximately.

Eight applications of insecticides were made at intervals of two weeks. Each application was carried out at a nominal dosage of 0.25 gallons per acre, which was equivalent to 0.20 lb. per acre of the p,p' isomer of DDT or 0.03 lb. per acre of the γ isomer of BHC.

In the area treated with DDT it is possible that both species of flies were eradicated for a short period, but small populations were re-established there by immigrant flies. In the other block the reduction was not so great, but it is not considered that this was due to a lesser effectiveness of the BHC, but to a combination of circumstances that led to less effective applications.

Some general observations are made upon the use of aircraft for this sort of work, particularly in connection with the effect of meteorological conditions.

Acknowledgements.

We are grateful to Messrs. Airwork Ltd. and their staff for operating the aircraft. The staff of the East African Tsetse and Trypanosomiasis Research and Reclamation Organisation gave much advice, and we have freely used techniques evolved by them. Much of the apparatus in the aircraft was designed and produced at the Chemical Defence Establishment, Porton, Salisbury, England.

The work has been directed by the Colonial Insecticides Committee, and has been paid for from the Colonial Development and Welfare Fund.

References.

- BURTT, B. D. (1942). *J. Ecol.*, **30**, p. 109.
FIEDLER, O. G. H. (1950). *Z. angew. Ent.*, **31**, pp. 509-536.
HOCKING, K. S., PARR, H. C. M., YEO, D. & ROBINS, P. A. (1953). *Bull. ent. Res.*, **44**, pp. 601-609.
HOCKING, K. S. & YEO, D. (1953). *Bull. ent. Res.*, **44**, pp. 589-600.
JACKSON, C. H. N. (1948). *Ann. Eugen.*, **14**, pp. 91-108.
SWYNNERTON, C. F. M. (1936). *Trans. R. ent. Soc. Lond.*, **84**, pp. 1-579.
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RESPONSES OF PESTS TO FUMIGATION.

III.—THE FUMIGATION OF WHEAT CONTAINING *CALANDRA* SPP.
(CURCULIONIDAE) WITH THREE FUMIGANTS, UNDER
REDUCED PRESSURE.*

By A. K. M. EL NAHAL.

Imperial College Field Station, Sunninghill, Berks.

A preliminary investigation of the responses of *Calandra* spp. to hydrogen cyanide was described by Salmond (1953). The work discussed in this paper extends the investigation to a critical study of three of the main procedures suggested for fumigation in chambers, namely fumigation at atmospheric pressure, sustained vacuum fumigation, and vacuum fumigation with simultaneous admission of air and fumigant. The methods used in these "vacuum fumigations" have been given a confusing variety of names. Those used in this paper follow the recommendations of Page & others (1953). Those features, such as methods of sampling the gas concentrations, which are primarily of chemical interest, including the sorption on wheat of the three fumigants used in this work when applied by the different methods, will be reported elsewhere (Nahal, in press). An investigation of the behaviour of methyl bromide in fumigations at reduced pressure has been reported by Brown and Heuser (1953).

This paper is concerned with the toxicity to adult *Calandra granaria* (L.) and *C. oryzae* (L.) of hydrogen cyanide, methyl bromide and ethylene oxide and the enhanced susceptibilities of the insects at reduced pressures. Changes in the method of applying the fumigant, or in the nature of the commodity in which the insects may be buried, may influence the apparent toxicity of the fumigant either by altering the concentration-time products to which the insects are subjected or by changing their susceptibility.

The experiments reported here were designed to distinguish between these two important components of toxicity.

Experimental.

The batches of test insects consisted of 40 adult *C. oryzae* and 40 *C. granaria*, without regard to sex, contained separately in muslin-topped 2 × 1-in. glass tubes together with sufficient English wheat (13 per cent. moisture content) to fill the tube. The breeding of the test insects, and the post-fumigation treatment of the insects, were as described by Salmond (1953) except that when mortality caused by ethylene oxide or methyl bromide was assessed, the complication of delayed mortality necessitated the extension of the post-fumigation observation period to 10 days. Parallel control batches of insects were used, to correct the estimated mortalities where necessary. One tube of each species was imbedded in the centre of a 1 cwt. sack of Manitoba wheat, of known moisture content. This sack was half filled by pouring the wheat into it and levelling it off, when the glass tube of test insects was laid in the centre together with the lead capillary tube used to take samples of gas from this position. In this way the natural packing of the grains was not disturbed by the subsequent insertion of the tubing after the filling of the sack had been completed. The whole sack

* Part of a thesis approved for the Ph.D. degree of the University of London.

was supported in a freely perforated bin, which was loaded by means of a hoist into a cylindrical steel fumigation chamber of 487 litres capacity, which has been briefly described by Brown (1936). A further pair of tubes containing test insects was suspended adjacent to the capillary tube used for sampling the gas in the free space around the sack.

In fumigations at atmospheric pressure and in sustained vacuum fumigations the fumigant was distilled directly into the chamber, but in vacuum fumigations with simultaneous admission of air and fumigant it was distilled into a much larger steel chamber of 5,520 litres capacity, as described briefly by Lubatti and Blackith (1950). When the air in this large chamber was found to contain the required concentration of fumigant, the smaller chamber containing the wheat was evacuated and the mixture of gas and air from the larger chamber allowed to enter it through a connecting pipe until the pressure approached atmospheric. The temperature in the smaller chamber, which was lagged by a circulating water system thermostatically controlled, was kept constant at $20^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. during any one fumigation. Gentle stirring in the free space inside the chamber was effected by a fan driven through a vacuum-tight bearing, and sorption was minimised by painting the inside of the chamber with a good oil paint.

The pump used to evacuate the chamber reduced the pressure to 4 cm. mercury in 5 min. and, when the pump was shut off by a valve, the pressure change in the chamber during the period of a vacuum fumigation (4 hours) was only 1 cm. mercury.

The wheat in the sack was weighed to the nearest 4 oz., a negligible variation on the standard weight of 1 cwt., and was so loaded that the cylindrical mass of grain to be penetrated by the fumigant was always 18 in. high and of 17 in. diameter. Samples of gas were taken 10 minutes after the fumigant was introduced, from the centre of the sack, from towards its perimeter ($4\frac{1}{2}$ inches from the surface of the wheat) and from the free space. Subsequent samples from these three positions were taken after $\frac{1}{2}$, 1, $2\frac{1}{2}$ and 4 hours, when the chamber was opened. Immediately before opening the chamber the grain was washed twice with air after each of the two methods involving reduced pressure had been used, by filling the chamber with clean air, evacuating to 10 cm. mercury, and refilling with clean air (Nahal, in press). Samples were taken after each air-wash to determine their effectiveness in rapidly removing the fumigant. The small additional concentration-time products to which the insects were exposed during these air-washes have been allowed for in the statistical analyses. No air-washes were done after fumigations at atmospheric pressure.

Design of the experiments.

The experiments were designed factorially, with the four factors (method of fumigation, moisture content of wheat, fumigant, and dose of fumigant), each at three levels (Table I). Other factors, period of exposure, temperature, etc., were kept constant throughout the series of 81 experiments specified by the design. The experiments were arranged in three blocks of 27 fumigations each, and a whole block was devoted to the exploration of the properties of each fumigant. After correction for any control responses, the responses of the weevils were subjected to the angular transformation, and an analysis of variance was applied to the transformed data.

Results and Discussion.

Previous work attempting the comparison of methods of "vacuum fumigation" has relied either on biological assessment by means of test insects (Lepigre, 1949) or on measurement of the free space fumigant concentration, which does not enable the concentration-time products attained inside the grain to be

estimated with sufficient accuracy (Young & others, 1935). In order to distinguish between the direct influence of the variable factors in this experiment on the susceptibility of the insects, and their indirect influence on mortality through

TABLE I.
Factorial arrangement of the experiments.

Factor				Level
Fumigant	Methyl bromide Ethylene oxide Hydrogen cyanide
Nominal dosage of fumigant (mg./l.)				Methyl bromide and ethylene oxide 6, 10, 14 Hydrogen cyanide 10, 18, 26
Moisture content of wheat		..		9% 13% 17%
Method of fumigation		Atmospheric pressure Sustained vacuum Vacuum fumigation with simultaneous admission of air and fumigant

changes induced in the available concentration-time products to which the test insects were exposed, the changes in components of the mortality comparisons attributable to the indirect influence, *i.e.*, to changes in concentration-time products, were removed from the data by an analysis of covariance. Any change in the fumigation conditions results in a change of mortality. For example, mortality is lower in the centre of a sack than in the free space, and so is the concentration-time product experienced by the insects. The change in concentration-time predicts a change in mortality which may be greater or less than that observed. The discrepancy between these observed and predicted mortalities is attributed to the direct influence of the different environments on the resistance of the test insects. Similarly, the direct effect of reduced pressure can be estimated by comparing the observed difference of mortality between atmospheric and sustained vacuum fumigations, in relation to the different concentration-time products.

Where the mortality of the insects changes mainly as a reflection of a change in the concentration-time product to which they were exposed the results will agree closely with those calculated from the results of chemical sampling. Apart from noting the satisfactory concordance of the chemical and biological results, there is little point in discussing this part of the biological information here, as to do so would be to repeat what has already been said about the chemical investigation (Nahal, *in press*). Of more immediate interest, however, are those changes in mortality which are not associated with changes in concentration-time product.

In all the experiments, even at atmospheric pressure, mortality among *C. oryzae* was higher than among *C. granaria*, which confirms the observations of Salmond (1953). The biological assessment of the fumigations confirms that vacuum fumigation of wheat with simultaneous admission of air and fumigant yields results at best equal to those obtained by fumigation at atmospheric pressure. Pressure reduction, unless sustained, does not influence mortality to any important extent.

The results of the covariance analysis for *C. granaria* are represented graphically in fig. 1, the first time, it is thought, that such representation has been attempted. The height of each column of the histogram indicates the relative magnitudes of the changes in mortality in response to a change in the experi-

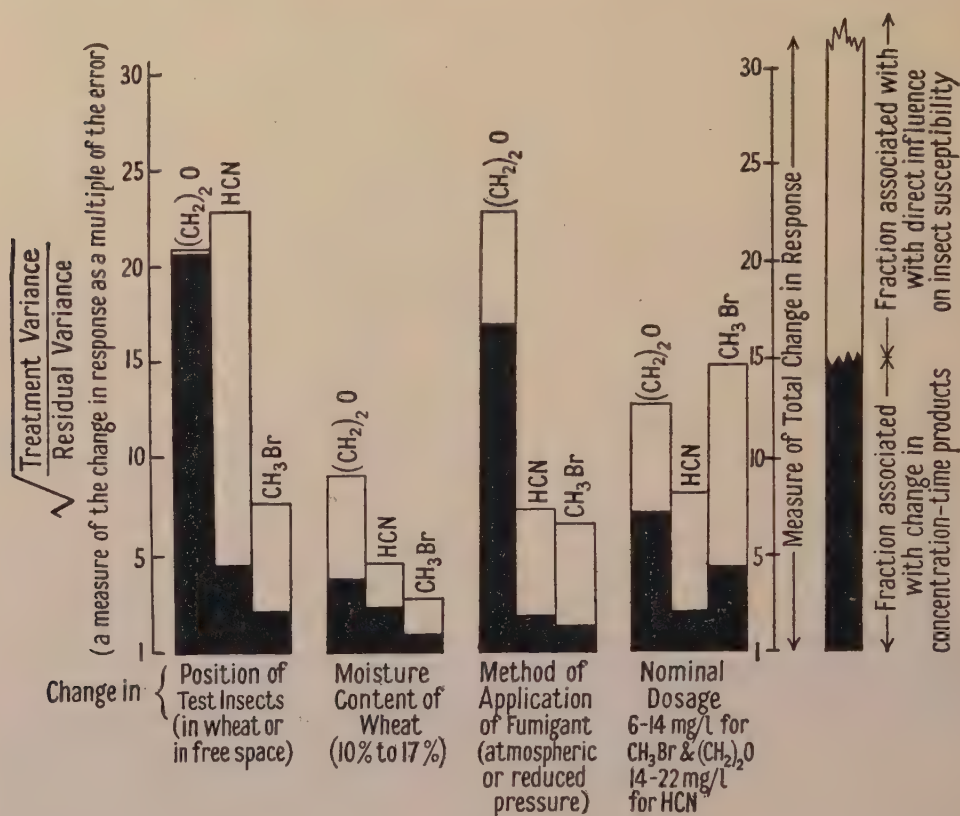


Fig. 1.—Relative importance of changes of fumigation conditions in altering the responses of *Calandra granaria*.

mental conditions either directly because the environment of the test insects (apart from the fumigant) has been altered, or indirectly because of the associated change in the concentration-time products at the position of the test insects.

For example, the first histogram in fig. 1 shows that the difference in the response of weevils inside or outside the sack is much less for methyl bromide fumigations than when the other two fumigants are used, a reflection of the much higher sorption and reduced powers of penetration of hydrogen cyanide and ethylene oxide. In the case of ethylene oxide, almost all the reduction of response of the weevils in the sack is caused by this delayed and reduced penetration of fumigant, and the substantial shaded area in the histogram reflects the consequent reduction of concentration-time products in the sack. When hydrogen cyanide is the fumigant, however, although the total reduction of response is similar (about 20–25 times the experimental error), a relatively small part is due to change in concentration-time product, and therefore the greater part must be associated with a lower mortality found when a given concentration-time product is presented in a sequence of concentrations beginning with rather low doses. This is represented quantitatively by the clear area of the appropriate column.

That sustained pressure reduction increases the "direct" component of mortality susceptibility only slightly more when ethylene oxide is used than for the other two fumigants is also shown in fig. 1 (histogram 3). A change of moisture content (histogram 2) of the wheat also affects the susceptibility of the weevils to ethylene oxide by altering the humidity of their environment. This alteration is less marked for hydrogen cyanide and methyl bromide. The substantial "direct" components of the mortality increments associated with an increase of nominal dosage (histogram 4) are also noteworthy. Such unexpected sources of variation constitute further examples of the breakdown of Haber's rule relating the transformed mortality to the logarithm of the product of concentration of gas and time of exposure, in addition to those which have already been noted both for preliminary and for sustained pressure reduction fumigations by Salmond (1953).

A more detailed analysis of the data discloses that when hydrogen cyanide is used as fumigant, the increase in response caused by sustained pressure reduction is lessened when the wheat has a high moisture content, even though the higher humidity by itself does not greatly influence susceptibility to this particular fumigant in the absence of pressure reduction. With ethylene oxide, however, not only does the higher moisture content of the grain diminish the effect of reducing the pressure, but it reduces the mortality of the insects even at atmospheric pressure. In methyl bromide fumigations, on the contrary, changes in moisture content of the wheat have no important effect on mortality with or without pressure reduction.

In the instances cited here, the shape of the concentration-time curve, as well as the total area bounded by this curve and the time-axis, are of importance. With curves which rise rapidly, that is when a high concentration of gas is rapidly built up round the insects as in the free space, the full toxicity of the hydrogen cyanide is available. When, however, the gas penetrates but slowly to those weevils in the centre of the sack, it seems likely that some form of additional resistance collectively termed protective stupefaction sets in which greatly diminishes the toxicity of the fumigant to the insects. Such differences in mortality have been ascribed by various authors to definite physiological mechanisms, but in each instance the interpretation of the results seems to be founded on nothing more than unverified speculation. The term "protective stupefaction" is used here solely in the descriptive sense, without causal implications. Protective stupefaction in this loose sense is well known to occur when hydrogen cyanide is used to fumigate some insects under certain conditions, but the illustration here is of particular importance.

Gray and Kirkpatrick (1929) were the first to observe that, under certain conditions, scale insects exhibit protective stupefaction if exposed to a preliminary sublethal dose of hydrogen cyanide. Lindgren (1938) also found that grain weevils exposed to low cyanide concentrations for 5 min. before a normal concentration was applied were much more resistant than untreated insects. In vacuum fumigations with simultaneous admission of air and fumigant, conditions like these may well occur when the fumigant penetrates by pressure differences and atmospheric pressure is restored by admitting the gas-air mixture. Such doses will soon disappear by sorption on the wheat and provide a low concentration-time product. They are followed later by the normal ingress of fumigant under a diffusion gradient. Consistent with this interpretation is the fact that when hydrogen cyanide is the fumigant, restoring the pressure during dosage gives notably lower mortality in the wheat-embedded weevils than even the atmospheric method of fumigation (fig. 4). Although the mortality reduction associated with protective stupefaction is not so marked when methyl bromide is used, it is still large enough to be of economic importance, as Table II discloses. In this Table, selected sets of experiments are recorded for which

roughly equal concentration-time products were attained in the free space and in the centre of the sack. In every instance, the mortality in the sack is materially lower than in the free space, although the insects should give nearly equal

TABLE II.

Responses of *C. granaria* to given concentration-time products of methyl bromide in the free-space or in sack of wheat.

Method	Concentration-time product (mg. hr./l.)	Percentage mortality of test insects	
		Free space	Centre of sack
Sustained vacuum fumigation ..	50.73	—	85
" " " ..	50.19	100	—
" " " ..	51.46	100	—
Sustained vacuum fumigation ..	21.57	—	74
" " " ..	21.37	86	—
" " " ..	21.66	90	—
Atmospheric fumigation ..	19.74	—	5
" " " ..	16.39	—	0
" " " ..	21.70	49	—
Atmospheric fumigation ..	30.59	—	32
" " " ..	36.21	100	—
" " " ..	35.01	95	—
Vacuum fumigation, with simultaneous admission of air and fumigant ..	21.73	—	15
" " " " " ..	23.94	51	—
Vacuum fumigation, with simultaneous admission of air and fumigant ..	32.39	—	42
" " " " " ..	38.61	98	—

responses if Haber's rule were valid for these experiments. In sharp contradiction to the virtually unsupported claims made for vacuum fumigation with simultaneous admission of air and fumigant (Lepigre, 1949) the disadvantages arising from the slow access of fumigant in atmospheric pressure fumigation to insects imbedded in grain are exaggerated when the former method is employed with methyl bromide. In Table II the responses recorded are those of *C. granaria*, because of the difficulty of so adjusting the conditions that graded responses should be obtained with both species. Throughout the work the conditions were made suitable for obtaining the maximum available information from *C. granaria*, the more resistant species. Detailed discussion of the interpretation of the mortality differences occurring when a given concentration-time product is applied in different ways will be deferred until a later paper in this series, when further evidence will be presented by other workers.

The relative effectiveness of the three different methods of fumigation is shown in figs. 2, 3 and 4, in each instance with a different fumigant. Each of the points represented on these graphs are the means of six determinations, and are averaged over the two species of *Calandra* and over the three moisture contents of the wheat. This procedure is justified by the orthogonality of the factors. These results show that the influence of sustained pressure reduction is different with the three fumigants used. The greatest enhancement of toxicity occurs when ethylene oxide is used, the improvement being relatively small with methyl bromide and hydrogen cyanide. With this last fumigant, however,

vacuum fumigation with simultaneous admission of air and fumigant gives inferior results compared with the atmospheric method, of which it is in other respects substantially the equal. Lepigre (1949) does not recommend the use of hydrogen cyanide when fumigating by this method. In all situations explored

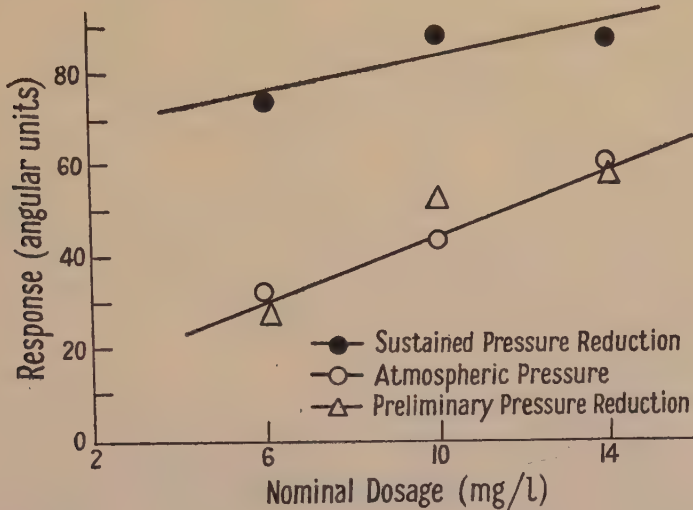


Fig. 2.—Responses of *Calandra* spp. to three methods of fumigation with ethylene oxide. (Preliminary pressure reduction is defined in the text as vacuum fumigation with simultaneous admission of air and fumigant: see also figs. 3 & 4.)

here, the method of sustained pressure reduction is superior to the other two, although when the fumigant is methyl bromide, which is only slightly sorbed, this superiority is less marked except at low doses of fumigant, becoming negligible at higher dosages.

Of the many changes brought about by the sustained pressure reduction, there is undoubtedly a direct enhancement of the susceptibility of insects to fumigation

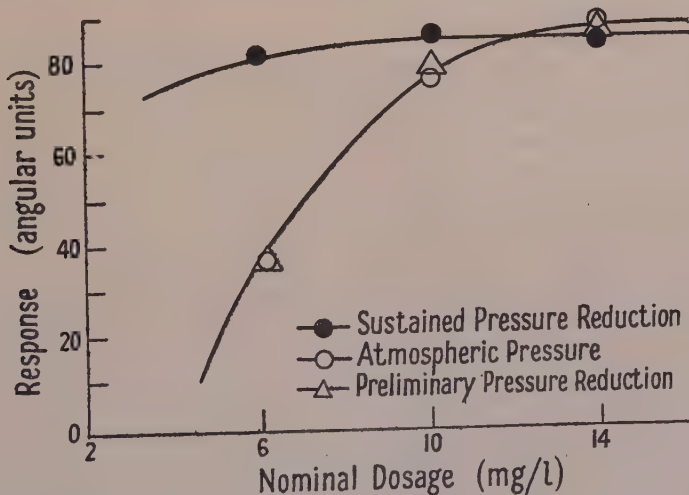


Fig. 3.—Responses of *Calandra* spp. to three methods of fumigation with methyl bromide.

(Moore & Carpenter, 1938) and it is thought probable that there is a component of the resultant mortality which may be attributed to the effect of the reduced pressure when no fumigant is applied. Investigations of these problems have been done and will be reported separately by various workers. It should then

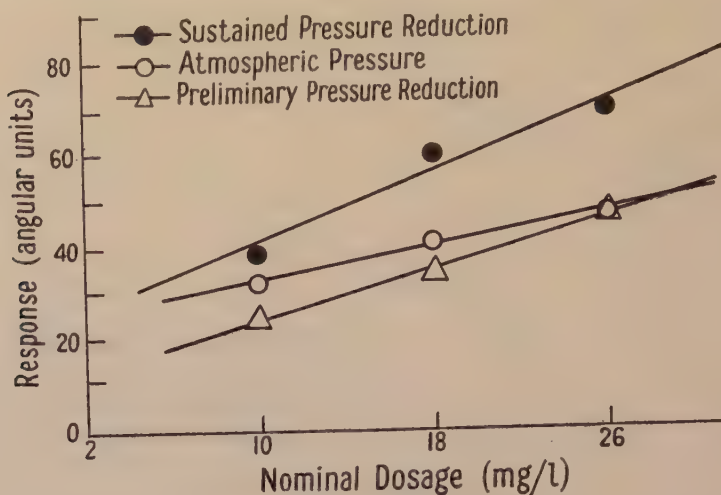


Fig. 4.—Responses of *Calandra* spp. to three methods of fumigation with hydrogen cyanide.

be possible to review the whole subject of fumigation under reduced pressure in the light of these investigations.

Summary.

Wheat of three moisture contents was fumigated with ethylene oxide, methyl bromide and hydrogen cyanide. Both chemical and biological assays were used to follow the changing distribution of the fumigant when applied with sustained vacuum, in a vacuum fumigation with simultaneous admission of air and fumigant, or at atmospheric pressure.

As these various factors were altered, in a multifactorial experiment, the changes in the fumigant distribution were also recorded chemically, and then analysed statistically. Changes in the mortality of both species of *Calandra*, either buried in a one-cwt. sack of wheat or in the free-space, were estimated so that that part of the changes which was associated with the altered fumigant distribution was segregated by a covariance analysis and the remainder formed an estimate of the direct influence of the experimental conditions on the susceptibility of the weevils to the fumigants.

When either ethylene oxide or methyl bromide is used as a fumigant for wheat, vacuum fumigation with simultaneous admission of air and fumigant gives results, in terms of the control of *Calandra* spp., which are almost indistinguishable from those obtained at atmospheric pressure under comparable conditions. When hydrogen cyanide is the fumigant, atmospheric fumigation is superior, quite apart from the capital expense and other practical disadvantages of fumigations at reduced pressures.

In fumigations with the more strongly sorbed ethylene oxide and hydrogen cyanide sustained vacuum fumigation is markedly an improvement over the other two methods. With the weakly sorbed fumigant methyl bromide, this advantage is only appreciable at low doses (6 mg./l.) and disappears at a nominal

dosage of 14 mg./l. Damp grain generally diminishes the advantages of sustained vacuum fumigation to some extent.

Apart from the total concentration-time product available to the test insects, the shape of the curve representing the increase of concentration with time seems to be important, thus constituting a further failure of Haber's rule for fumigant toxicity. High concentrations applied for short periods are more toxic than low concentrations applied over a longer period.

When methyl bromide, or hydrogen cyanide, is applied in a way which admits small sublethal doses to the grain before the lethal dose penetrates it, the resistance of the test insects is much enhanced and this change is termed "protective stupefaction" until the nature of the phenomenon is clarified by further evidence. Although sufficiently marked to be of economic importance with methyl bromide, the supposed protective stupefaction is most serious when hydrogen cyanide is the fumigant. Where sorption reduces the rate of penetration of hydrogen cyanide to weevils buried in wheat this phenomenon may lower the response to the fumigant five times as much as the lessened response when sorption reduces the corresponding concentration-time product.

Acknowledgements.

The writer is indebted to Professor J. W. Munro for facilities to undertake this investigation during the tenure of a study mission from the Government of Egypt, which is gratefully acknowledged. The covariance analysis and its representation in fig. 1 were, like the statistical analyses throughout this series, by Dr. R. E. Blackith. The writer is particularly grateful to Dr. A. B. P. Page for his advice and encouragement throughout this work.

References.

- BROWN, W. B. (1936). Determination of fumigants VI. Purity of commercial ethylene oxide in cylinders.—*J. Soc. chem. Ind.*, **55**, pp. 321T–325T.
- BROWN, W. B. & HEUSER, S. G. (1953). Behaviour of fumigants during vacuum fumigation. I. Penetration of methyl bromide into boxes of dates.—*J. Sci. Fd Agric.*, **4**, pp. 48–57.
- CRUMB, S. E. & CHAMBERLAIN, F. S. (1933). A comparison of the effectiveness of sustained vacuum and dissipated vacuum in fumigation with hydrocyanic acid gas.—*J. econ. Ent.*, **26**, pp. 259–262.
- GRAY, G. P. & KIRKPATRICK, A. F. (1929). The protective stupefaction of certain scale insects by hydrocyanic acid vapour.—*J. econ. Ent.*, **22**, pp. 878–892.
- LEPIGRE, A. L. (1949). La déinsectisation par fumigation avec vide préalable.—*Docum. phytosanit. Minist. Agric. Fr. Sér. ent.*, no. 9, 818 pp.
- LINDGREN, D. L. (1936). Vacuum fumigation.—*J. econ. Ent.*, **29**, pp. 1132–1137.
- LINDGREN, D. L. (1938). The stupefaction of red scale, *Aonidiella aurantii*, by hydrocyanic acid.—*Hilgardia*, **11**, pp. 211–225.
- LUBATTI, O. F. & BLACKITH, R. E. (1950). Fumigation of agricultural products II. The susceptibility of seed potatoes to the vapour of methyl bromide.—*J. Sci. Fd Agric.*, **1**, pp. 240–244.
- MOORE, W. & CARPENTER, E. L. (1933). The fumigation of insects with hydrocyanic acid: effect of different air pressures.—*J. econ. Ent.*, **31**, pp. 419–426.

- PAGE, A. B. P., BLACKITH, R. E., BROWN, W. B. & HEUSER, S. G. (1953). Descriptive terms for vacuum fumigation.—Chem. & Ind., **1953**, pp. 353–354.
- SALMOND, K. F. (1953). Responses of pests to fumigation. II. Toxicity of hydrogen cyanide to *Calandra* spp. under reduced pressure.—Bull. ent. Res., **44**, pp. 225–230.
- YOUNG, H. D., WAGNER, G. B. & COTTON, R. T. (1935). The vacuum fumigation of flour products with hydrocyanic acid.—J. econ. Ent., **28**, pp. 1049–1055.
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RESPONSES OF PESTS TO FUMIGATION.

IV.—THE RESPONSES OF *CALANDRA* SPP. TO REDUCED PRESSURES.*

By A. K. M. EL NAHAL.

Imperial College Field Station, Sunninghill, Berks.

Nahal, in the paper immediately preceding this, laid emphasis on the desirability of investigating the several components of toxicity associated with fumigation under reduced pressure. The influence on the susceptibility of test insects of such variable factors as the method by which the fumigant is applied can hardly be assessed until the changes induced in the fumigant concentration-time products available to the test insects have been investigated by chemical methods. In the same way, the influence of pressure reduction on the susceptibility of insects to a fumigant cannot properly be estimated until the mortality of insects due to reduced pressures has been determined when no fumigant is used. Such an investigation is the subject of this paper.

Previous workers, for example Cole (1905) working with *Calandra oryzae* (L.) and Fisk and Shepard (1938) using *Tribolium confusum* Duv., found that the death of insects at reduced pressures was accelerated in the absence of external sources of moisture, and that one of the most important results of pressure reduction was the rapid, and often fatal, failure of the insects to maintain their water-balance. Govaerts and Leclercq (1946) have summarised the evidence that strongly suggests a rapid exchange between moisture in the atmosphere and in insects, leading to the complete replacement of the water content of the insects within a few days at atmospheric pressure. A close association between loss of water and the mortality of insects has been noted by Livingstone and Reed (1940).

Experimental.

The test insects used in this work (*Calandra granaria* (L.) and *C. oryzae*) were bred and handled as described in Part III except that the batches consisted of 30 adults of either species which were placed in the muslin-topped tubes without wheat or other food but supported by a piece of filter paper.

These tubes were placed in glass chambers, of the kind described by Turtle (1941) which have a capacity of about 1 litre and are as used by Salmond (1953).

In order to ensure that the required amount of water was present in the chambers, apart from that contributed by the test insects, sealed glass ampoules containing weighed amounts of water were introduced into the chambers and these were then filled with dry air as described by Salmond. When the chambers had been sealed, and the required pressure reduction established, these ampoules were broken by shaking the chamber, which was then kept in an incubator at the temperature and for the period of exposure specified by the experimental design (Table I). Post-evacuation treatment and inspection for mortality were described in Part III, above.

Design of the experiments.

Each of the four factors (pressure reduction, temperature, exposure period and amount of moisture in the atmosphere) were investigated at three levels, and

* Part of a thesis approved for the Ph.D. degree of the University of London.

the values at which these levels were set in the two series of experiments are given in Table I. The more complicated interactions between the factors were not thought likely to be important in these experiments, which were on a much

TABLE I.
Factorial arrangement of the experiments.
First series of experiments

Factor	Symbol	Levels chosen
Pressure (cm. mercury)	P	2, 4, 8
Water vapour (mg./l.)	W	18, 22, 26
Exposure period (hr.)	E	5, 6.5, 8
Temperature (°C.) ..	T	20, 24, 28

Second series of experiments

T and P as for 1st series.	W set at 0, 22, excess.
	E ,, ,, 1, 2.25, 5.

smaller scale than the very full examination of reduced pressure fumigation described in Part III. The nine treatment combinations indicated by Table I were therefore given a preliminary examination by arranging them in a Graeco-Latin square design and allotting one tube of 30 *C. granaria* and one of *C. oryzae* to each combination at random.

Results.

In the first series of experiments there were four complete replications, and a joint analysis of variance was done on the angular transforms of the percentage mortalities obtained. The combined residual variance, consisting of unisolated interactions, was tested for bias associated with the presence of significant interactions between factors by comparing it with the difference between replicates, which provided an unbiased if insensitive measure of experimental error. The two measures agreed within sampling limits and were pooled to give a more sensitive error variance against which the main factors were tested.

Over the short ranges examined, neither the exposure periods nor the amount of water present in the atmosphere influenced the death of the insects, though both factors approached significance at the 5 per cent. level. *C. oryzae*, as noted before, was in all cases much more easily killed than *C. granaria* (Salmond, 1953; and Nahal, Part III, above). The proportion of insects dying increased both with increase in temperature (Table II) and a decrease in pressure (Table III).

TABLE II.
Mean percentage mortalities of *Calandra* spp. at low pressures, with increasing temperatures.
(Averaged across all other factors listed in Table I.)

Temp. °C.	Mortality of	
	<i>C. oryzae</i>	<i>C. granaria</i>
20	38	1
24	59	2
28	84	10

Each figure is the mean of twelve observations.

A second series of experiments was then carried out with three complete replications of an arrangement similar to that described above. For this second series, however, the range of atmospheric moisture was considerably extended

TABLE III.

Mean percentage mortalities of *Calandra* spp. with decreasing pressure. (Averaged across all other factors in Table I.)

Pressure (cm. mercury)	Mortality of	
	<i>C. oryzae</i>	<i>C. granaria</i>
8	4	1
4	78	2
2	97	10

Each figure is the mean of twelve observations.

and the range of duration of treatment altered. In the first series the amounts of water present were always insufficient to saturate the atmosphere, which at 28°C. and at atmospheric pressure can hold 26.9 mg./l. of water (Oxley, 1948). In the second series, dry air was used in place of the lowest moisture level previously adopted, and where the highest amount of water added during the first series was 26 mg./l. excess moisture was present throughout the corresponding experiments of the second series. It was thought that the responses of the weevils could not remain independent of the duration of treatment if this quantity were indefinitely decreased, so that a lower range, contiguous to that used in the first series of experiments, was adopted (Table I).

Under these more informative conditions, the enhanced susceptibility of *C. oryzae* to both temperature and pressure changes was further substantiated, and, in addition, the mortality of both species was shown to decrease as the exposure period is shortened (Table IV). Of more importance, however, is the greatly reduced mortality of both species when excess moisture is present in the atmosphere (Table V), a result which agrees with the observations of Livingstone and Reed (1940).

TABLE IV.

Mean percentage mortalities of *Calandra* spp. at low pressures, with decreasing periods of exposure. (Averaged across all other factors in Table I.)

Exposure period (hr.)	Mortality of	
	<i>C. oryzae</i>	<i>C. granaria</i>
1.00	20	1
2.25	47	2
5.00	43	2
6.50	56	1
8.00	67	9

These figures are combined from both series of experiments, so that for 1 and 2½ hr. each is based on nine observations, for 5 hr. it is based on 21 observations and for 6.5 and 8 hr. the means of twelve observations are recorded.

This atmospheric moisture is not expressed here as a relative humidity because the amount of water lost from the insects under the experimental conditions is large enough to add considerably to the humidity. All that Table V

TABLE V.

Mean percentage mortalities of *Calandra* spp. at low pressures, with changing moisture content of atmosphere. (Averaged over all other factors.)

Atmospheric moisture (mg./l.)	Mortality of	
	<i>C. oryzae</i>	<i>C. granaria</i>
0 (dried) ..	83	1
22	57	12
Excess (saturated)	3	1

Each figure based on nine observations.

records is the added weight of water per litre of air. The distinction is of importance because the size of the chamber in which the experiments are carried out must influence the results. With the small one-litre chambers (Turtle, 1941) used in this work the loss of water to the atmosphere by the test insects will materially reduce further desiccation, whereas in large fumigation chambers the influence of evaporated water will be negligible, if the chamber be empty, and dependent on the moisture content and nature of the commodity in a loaded chamber. In the more extensive investigation of the problems which are here discussed in a preliminary way, and are to be described in another paper, the moisture content of the experimental atmosphere was stabilised at a predetermined level.

Discussion.

Experiments in 1-litre glass chambers (Turtle, 1941) confirm that at pressures between 2 and 8 cm. mercury there is a considerable mortality of *C. oryzae* and to a lesser extent of *C. granaria* in the absence of fumigant. This mortality is partly suppressed by high humidities, and enhanced by a rise in temperature, changes which may also influence the susceptibility of the insects to a fumigant.

Indeed, any change in the conditions of a fumigation under reduced pressure may materially influence mortality in a number of ways. To give only one of many possible examples, an increase in the moisture content of wheat from, say, 9 to 17 per cent., in a fumigation under reduced pressure, may have the following results:—

- (1) Reduction of the concentration-time product available in the bulk of the commodity, by increasing the sorption of the fumigant (Nahal, in press).
- (2) Change of rate of penetration of the fumigant into the commodity so that when methyl bromide or hydrogen cyanide are used protective stupefaction of the weevils is enhanced. (Nahal, Part III, above.)
- (3) Increase in the amount of fumigant retained by the wheat and also in the fraction of the total amount sorbed which is chemically combined with the wheat (Lubatti & Harrison, 1944; Nahal, in press). This change is distinguishable from (1) in that for low vapour-pressure fumigants such as the γ isomer of BHC, the sorbed fumigant can act as a contact poison. (Nasir, in press.)

- (4) Influence directly the susceptibility of the weevils to methyl bromide, hydrogen cyanide, and in particular to ethylene oxide (Nahal, Part III, above; Fisk & Shepard, 1938).
- (5) Diminution of the responses of the weevils, particularly *C. oryzae* to low pressures by raising the relative humidity in the intergranular spaces from 30 to 80 per cent. (Table V). The humidity data are from Oxley (1948).

Of these varied effects, (1), (2) and (5) will tend to decrease the mortality, (4) will tend to increase it and the part played by (3) though likely to be small, is not accurately known. This illustration is given to emphasise that only well-designed experiments in which chemical and biological measurements are used together, and are capable of assessment by statistical methods, can provide the data necessary to unravel the complicated situations which arise during fumigation at low pressures. Lepigre's (1949) claims for vacuum fumigation with simultaneous admission of air and fumigant are an example of the danger of substituting plausible hypotheses and unplanned observations of mortality for systematic experimentation.

In the work reported here, the pressure has not been reduced below 2 cm. mercury, and in view of Moore and Carpenter's (1938) observations of a sharp decrease of mortality when some insects are exposed to extremely low pressures the conclusions drawn from the above experimental results should not be extended outside the ranges actually investigated. For commercial vacuum fumigation practice such extension is unnecessary, 4 cm. mercury and upwards being representative values for such work. Control of pests by reduced pressure alone is rarely the object of such practices, although Bare (1948) suggests that ten-day exposure to a pressures of 28 cm. mercury should control all stages of *Lasioderma serricorne* (F.) in tobacco bales without fumigation. Changes in penetration and sorption will be more important in the short exposures of from one to two hours, usually adopted in fumigation under reduced pressure, although for species such as *C. oryzae* which seem consistently susceptible to low pressures, appreciable mortality from the reduced pressure is to be anticipated (Table IV).

The relatively large changes in response not associated with changes in the concentration-time products attained inside the sack of wheat, as shown in fig. 1 of Part III, which were found when fumigation at reduced pressures was compared with that at atmospheric pressure, seem insufficiently accounted for by the mortalities found in the present experiments, although a direct comparison is difficult. The results suggest a major influence of reduced pressures on the susceptibility of the insects to fumigants in addition to the direct actions of the low pressures and concentration-time products of the fumigant separately.

Summary.

The direct action of reduced pressures, in the absence of any fumigant, on *C. oryzae* and *C. granaria* ~~have~~ been investigated in glass chambers of about 1 litre capacity. *C. oryzae* is sufficiently sensitive to make the direct effect of reducing the pressure within the range 2 to 10 cm. mercury of some practical importance, but *C. granaria* is more resistant. Mortality of both species increases for exposure periods of up to 8 hr. and for increased temperatures from 20°C. to 28°C. A large part of the observed mortality must be associated with enhanced water-loss from the insects at reduced pressures, as little response occurs when the atmosphere is kept saturated with water-vapour. /has

Acknowledgements.

The acknowledgements made in Part III, the paper immediately preceding this, apply equally here.

References.

- BARE, C. O. (1948). The effect of prolonged exposure to high vacuum on stored-tobacco insects.—*J. econ. Ent.*, **41**, pp. 109–110.
- COLE, F. J. (1906). The bionomics of grain weevils.—*J. econ. Biol.*, **1**, pp. 63–71.
- FISK, F. W. & SHEPARD, H. H. (1938). Laboratory studies of methyl bromide as an insect fumigant.—*J. econ. Ent.*, **31**, pp. 79–84.
- GOVAERTS, J. & LECLERCQ, J. (1946). Water exchange between insects and air moisture.—*Nature, Lond.*, **157**, p. 483.
- LEPIGRE, A. L. (1949). La désinsectisation par fumigation avec vide préalable.—*Docum. phytosanit. Minist. Agric. Fr., Sér. ent. no. 9*, 318 pp.
- LIVINGSTONE, E. M. & REED, W.B. (1940). Water vapour as a factor affecting the survival of *Ephestia elutella* and *Lasioderma serricornis* at reduced pressure.—*Ann. ent. Soc. Amer.*, **33**, pp. 583–587.
- LUBATTI, O. F. & HARRISON, A. (1944). Determination of Fumigants XVII. Comparison of the sorption of hydrogen cyanide, ethylene oxide, trichloroacetonitrile, and methyl bromide by wheat.—*J. Soc. chem. Ind.*, **63**, pp. 353–359.
- MOORE, W. & CARPENTER, E. L. (1938). The fumigation of insects with hydrocyanic acid: effect of different air pressures.—*J. econ. Ent.*, **31**, pp. 419–426.
- OXLEY, T. A. (1948). The scientific principles of grain storage.—Liverpool, North. Publ. Co.
- SALMOND, K. F. (1953). Responses of pests to fumigation. II. Toxicity of hydrogen cyanide to *Calandra* spp. under reduced pressure.—*Bull. ent. Res.*, **44**, pp. 225–230.
- TURTLE, E. E. (1941). Studies in the retention of hydrogen cyanide by certain products on fumigation.—Ph.D. Thesis, University of London.
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STUDIES ON *THERAPTUS* SP. (COREIDAE); THE CAUSE OF THE GUMMING DISEASE OF COCONUTS IN EAST AFRICA.

By M. J. WAY.

*Clove Research Scheme, Zanzibar.**

E.H.N.

(PLATE XI.)

Widespread damage to coconut fruits in British East Africa has been reported by McDonald (1925) and by Welsford (1925, 1926); this became known as the gumming disease or gummosis of coconuts and was attributed to unfavourable soil conditions. Recent work has shown, however, that a hitherto unknown species of *Theraptus* (Coreidae) is responsible (Way, 1951). Damage by this insect is negligible in coconut palms occupied by the tree-nesting ant *Oecophylla longinoda* (Latreille); work on the influence of the latter on *Theraptus* sp. is reported elsewhere (Way, 1951, 1953).

This paper gives the results of work on the distribution, biology and effect on the coconut palm of *Theraptus* sp.

The characters used at present for defining species of the genus *Theraptus* indicate that those collected in Kenya, Pemba Island and Zanzibar Island are all new and different species. However, the taxonomy of *Theraptus* and related genera is unsatisfactory, and it is considered unwise to describe these until the systematics of the group have been revised (W. J. Hall, priv. comm.). Revision is not possible at present, and thus in this paper the term "*Theraptus*" will be used to define all individuals of the genus *Theraptus* which were found attacking coconuts in British East Africa. Regardless of their origin, they all cause identical damage to coconuts.

Distribution in Coconut-Growing Areas.

In British East Africa coconut palms are grown mainly in a 10-20 mile wide belt along the Kenya and Tanganyika coastline, and also on the islands of Zanzibar, Pemba and Mafia. Small isolated plantations, of relatively little importance, occur inland.

In Kenya, plantations were examined along a 40 mile strip of coastline in the Mombasa area. With one exception (at Shanzu Bay) typical *Theraptus* damage was found in all of them. At Gazi, where damage was severe, twelve nymphs of *Theraptus* were collected in the coconut palms; it was reported that affected palms were also common in the Malindi area 60 miles north of Mombasa.

In Tanganyika, much *Theraptus* damage was seen near Tanga and also along a 60 mile strip of coast in the Bagamoyo and Dar-es-Salaam areas. The most serious occurred in plantations of good palms at Chambezi, Mbagalla, and near the Morogoro road about 11 miles west of Dar-es-Salaam. Many of the palms bore no developing nuts and the rest all bore some damaged ones. The areas where serious *Theraptus* damage was observed were populated by the ant *Anoplolepis custodiens* (F. Sm.) which destroys the predatory *O. longinoda* but is itself not predatory on *Theraptus* (Way, 1953). The main coconut belt extends inland from Dar-es-Salaam for about 13 miles and, from here to Morogoro (about 150 miles inland), the small isolated plantations showed no *Theraptus* damage.

* Seconded from Rothamsted Experimental Station, Harpenden, Herts.

A total of 20 nymphal and adult *Theraptus* was caught on coconut palms in the Tanga, Bagamoyo and Dar-es-Salaam areas. Damage was also reported (G. Swaine, priv. comm.) from Kisiju (60 miles south of Dar-es-Salaam) and at Lindi near the Mozambique border. It is of interest that *Theraptus* sp. is apparently absent in some large plantations in northern Mozambique (M. Boesch, priv. comm.).

On Mafia Island, coconuts are grown in the isolated patches of fertile soils which occur amongst the scrub and heath vegetation. Fourteen nymphs and adults of *Theraptus* were caught in one plantation at Ngombeni where much damage was seen, but in others, few palms were damaged. The scarcity of *Theraptus* here was not due to predation by *O. longinoda* which was uncommon. *Theraptus* was absent on Bweju Island (1-2 miles long and about 100 yards wide) which lies between Mafia Island and the mainland, and is thickly planted with coconut palms.

The Zanzibar Protectorate includes the islands of Zanzibar and Pemba, coconut palms being most abundant on the former. *Theraptus* damage was seen in plantations throughout Zanzibar Island and was particularly severe around Zanzibar town, where the non-predatory ants, *Anoplolepis custodiens* and *A. longipes* (Jerd.), have destroyed the predatory *O. longinoda* (Way, 1953). On Pemba Island, where parts of all the main coconut-growing areas were examined, damage was widespread, being most severe to the north and north-east of the island. Many nymphs and adults of *Theraptus* were caught on coconut palms in both islands.

These surveys, although incomplete, suggest that *Theraptus* is widespread in the main coconut-growing areas of British East Africa.

Life Cycle.

Experiments on the life cycle of *Theraptus* collected in Zanzibar Island were conducted in an outdoor insectary with wire gauze walls and a roof that excluded sunlight. Individuals were caged with 1-4-month-old coconut palm spadices which were changed twice weekly. The experiments lasted from June to October 1951 during which the mean weekly temperature in the insectary (as shown by a recording thermograph) varied from 22.8-26.3°C., the mean throughout the period being 24.5°C. The recorded lengths of the various stages are shown in Table I.

TABLE I.

The lengths of stages in the life cycle of *Theraptus* at a mean temperature of 24.6°C.

Stage	Egg	Nymphal instars					Adult	
		1st	2nd	3rd	4th	5th	♂	♀
No. of individuals	6	6	6	6	7	7	3	3
Duration of stage (days)	8.5 (8.9)	3.5 (3.4)	6.5 (5.7)	7 (7)	6.5 (5.8)	9 (8-10)	83.5 (83-84)	53(45-66)

It can be seen that there are five nymphal instars, the mean length of time from egg laying to emergence of the adult being 41 days at 24.6°C. These data were obtained in the cool season, and there is evidence that development is quicker in the hot season, when the mean shade temperature rises above 28°C. Sunlight was excluded in these experiments and thus development was probably slower than in the field where the insect is exposed to isolation (Way, 1953).

Thus *Theraptus* which breeds continuously on the coconut palm, probably has over nine generations each year.

The virgin female *Theraptus* will lay sterile eggs but if mated may lay fertile eggs by the time she is nine days old. One pair of adults was seen copulating three times during the life of the female; a female, isolated for a few weeks after copulation, began to lay sterile eggs but after the male had been re-introduced resumed laying fertile ones. Once, when the female of a pair had died, a newly emerged female was caged with the old male who fertilised her successfully.

Theraptus lays eggs singly; in the insectary most were laid on the wire gauze of the cages and a few on the flowers or young nuts and in cavities on the stalks of the coconut spadix. The numbers of eggs laid by four females varied from 40-122 with a mean of 74 per female.

On coconut palms in the field, eggs were found only twice, each time in a crack on the underside of the inner spathe of an approximately 6-week-old spadix; 1st instar nymphs were also seldom seen. In the Solomon Islands, eggs and young nymphs of the closely related *Amblypelta cocophaga* China are reported to have been found on young leaves of the palm spike (Phillips, 1940) but, in Zanzibar, *Theraptus* nymphs were found only on or near the spadices.

Nature of Damage to Coconuts.

In Zanzibar the only part of the palm attacked is the spadix, and the vigour of the tree is not noticeably affected. Le

A newly opened spadix (Pl. XI, fig. 1) consists of a main stem whose branches bear the male and female flowers. The male flowers open within 2-4 weeks, just before the female, and pollination usually takes place after about 5 weeks. The number of female flowers per spadix varies from zero to over 200 depending on:—

- (a) the age of the palm; young or senescent palms bear none or few per spadix.
- (b) the variety of palm.
- (c) the season of the year. In Zanzibar, spadices bear more female flowers in the first half of the year than in the second half (see fig. 2).

The female flower is surrounded by bracts except at the tip where the stigma projects. The meristem is basal and as the nut elongates it projects beyond the ensheathing bracts. "Natural" fall of a varying proportion of young nuts occurs when they are about 5-10 weeks old. The nut is full sized after 5-6 months, but is not mature until after 11-13 months. After 5 months, premature nutfall rarely occurs and thus it is permissible to assess the eventual yield of mature nuts by counting them when 6 months old.

New spadices are produced at intervals of about 24 days throughout the year, and thus a healthy undamaged palm always bears about 15 spadices showing successive stages in the development of the nuts.

On a newly opened spadix *Theraptus* may feed at the base of the male flowers, and on the main stem and branches which at this stage are fairly succulent. The visible damage is a small superficial brown spot surrounding each feeding puncture which apparently does no harm to the spadix. For the later instars and adults the preferred feeding sites are the immature and mature female flowers and the young nuts. The stylets are inserted through the bracts and into the soft tissue of the underlying ovary or nut, each puncture causing a sunken necrotic area (Pl. XI, fig. 2). This, apparently, is not due to invasion by pathogenic organisms but to toxic saliva.

The severity of damage to the female flower depends on the age of the insect, the number of feeding punctures, and the age of the flower or nut at the time of attack. It was found that a single feeding puncture by a later instar nymph or

adult invariably destroys flowers or young nuts up to about eight weeks old. Dead flowers generally remain attached to the spadix for several months, but nuts older than about five weeks generally abscise and fall within a week. Single feeding punctures may cause premature fall of 8-12-week-old nuts but not of those older than about 12 weeks. Such damaged nuts develop to maturity. Ten or more feeding punctures by nymphal or adult *Theraptus* generally cause premature fall of nuts up to 12 weeks old, but those older than 16 weeks continue to develop even if severely damaged. As the nut increases in length, the necrotic areas caused by the punctures emerge from beneath the bracts and appear as a ring of lesions from which much gummy material exudes (Pl. XI, figs. 3 and 4)—hence the name “gumming disease”. The lesions widen as the nut expands, and sometimes become deep splits in the exocarp. Often a damaged nut becomes distorted during subsequent growth and a long scar is formed due to destruction of part of the meristematic tissue at the base of the nut (Pl. XI, fig. 5).

A damaged 8-16-week-old nut, if not immediately destroyed, continues to grow but if a deep lesion penetrates to the endocarp it bursts under internal pressure and the developing endosperm is destroyed; the exocarp remains alive, and the nut does not fall prematurely (Pl. XI, fig. 5). Otherwise the damaged nuts develop to maturity but are usually undersized and give low copra yields (Way, 1951 and Pl. XI, figs. 4 and 5).

Theraptus may also attack full-sized nuts (5-6 months old), the necrotic areas being visible when the bracts are removed. These nuts develop normally, and the copra yield appears to be unaffected. Feeding punctures are also made on the exposed surface of older nuts and each causes a slight wrinkle or pit; such damage is of no significance. In the insectary, both nymphs and adults fed and developed normally on nuts varying in age from 6-24 weeks; experiments with older nuts were not carried out. Adults were caged with pairs of spadices of different ages cut from the same tree and counts of feeding punctures showed that with nuts varying in age from 6-24 weeks there was no marked preference for a particular age.

“Premature nutfall”, caused by *Amblypelta cocophaga* China in the Solomon Islands, has been described briefly by Phillips (1940) and in detail by O'Connor and Leach (O'Connor, 1950). *A. cocophaga* is nearly related to *Theraptus* and the type of damage caused by the two insects appears to be identical.

Damage in Relation to *Theraptus* Population.

Table II shows that the 1st instar of *Theraptus* rarely causes noticeable damage; necroses made by 2nd instar nymphs are small and probably relatively harmless, but they become larger, and more numerous as the insect grows older.

TABLE II.

Results of insectary experiments showing the mean numbers of necrotic areas made in young coconut fruits by each nymphal instar and by a pair of adult *Theraptus*.

Instar	1st	2nd	3rd	4th	5th	Adult ♂ & ♀
Mean no. of nuts damaged			0.4	5	8	9	12	124
Mean no. of necrotic areas			0.6	13	24	26	34	346

A coconut palm produces an average of about 400 flowers each year. A single *Theraptus* during its lifetime makes about 200 feeding punctures, and, as a single

puncture can destroy a flower or young nut, it is apparent that even a very low population may cause serious damage. Field evidence confirms this for even in severely damaged plantations, the maximum number of *Theraptus* found on a single palm rarely exceeded two, and frequently none could be seen. In plantations where damage was most severe, the spadices were attacked soon after opening, and, in many palms, all the female flowers or nuts were destroyed before they were 8 weeks old (i.e. before they were capable of withstanding even a single feeding puncture). It was concluded that much movement of the adult *Theraptus* population was occurring in such plantations, because on many palms all flowers or nuts were destroyed before a new spadix had opened, thus necessitating migration to other palms which bore recently opened spadices.

In plantations where damage was less severe, some nuts reached a relatively resistant age without being attacked. These older damaged nuts may not fall prematurely but may remain as a source of food for many months. Thus migration of *Theraptus* from tree to tree is probably less frequent. These observations suggest that crop loss is not necessarily related directly to density of pest population. Thus, once all developing nuts in a block of palms have been destroyed, a relatively low population is probably sufficient to destroy all female flowers as they appear. By contrast, a relatively high population may do less harm to palms bearing older nuts capable of withstanding much feeding damage.

Both on the mainland and in the Zanzibar Protectorate it was noticeable that, apart from areas densely populated by the predatory *O. longinoda*, damage was most severe in plantations consisting of almost pure stands of closely planted palms; for example the Government plantations in Zanzibar Island. Where the palms are more widely scattered, as they are through clove plantations, damage was less severe. Decrease in severity of damage, with decrease in density of the host plant, is generally true of other pest problems but is particularly noticeable here probably because the effect is exaggerated by the unusually low density of the pest even when the host plant density is high.

Assessment of Crop Loss.

Although *Theraptus* damage is widespread, and although in many plantations it is associated with low yields, the extent of crop loss is difficult to assess because the full yielding capacity of the palms is unknown.

In Zanzibar Island, the Government plantation at Chumbuni yielded an average of 8 nuts per bearing palm during the years 1949-1951. In this plantation the predatory ant *O. longinoda* was absent (having been exterminated by the non-predatory *A. longipes*), and much damage to flowers and nuts was observed. In most Zanzibar Government plantations some palms, colonised by *O. longinoda*, are protected from attack, and although the uncolonised palms are usually badly damaged the average nut yield is higher; for example, the plantations at Kidichi yielded 30.8 nuts per bearing palm per year during 1940-1949. A plantation on Mafia Island where *Theraptus* is rare and damage slight, has been yielding over 80 nuts per palm per year, although the palms appeared no healthier than those in most Zanzibar Government plantations.

Palms occupied by *O. longinoda* are seldom damaged and in one experiment lasting throughout the year 1951 these palms yielded an average of 72.3 nuts compared with 13.4 from neighbouring unprotected palms (Way, 1953). Also, destruction of *O. longinoda* colonies in palms by invading *A. longipes* caused a fall in nut yield from about 6 to 1 per spadix as a result of *Theraptus* invasion. These data give some indication of the extent of crop loss caused by this insect.

An assessment of the loss due to *Theraptus* was made in part of a Zanzibar Government Plantation at Mazizini. Here the ant *A. longipes* was abundant, *O. longinoda* being absent. Most palms were in good condition but *Theraptus*

damage was severe, the mean nut yield varying from 5.3–8.6 per annum during 1949–1951. Two neighbouring blocks of 48 palms were chosen, one of which was untreated and acted as a control. In the other, hand dusters were used to treat the spadices of all palms with 0.4 per cent. γ BHC dust once a fortnight for 6½ months. The rate of application averaged 20 lbs. per acre, i.e., rather less than ½ lb. per palm. A “guard row” surrounding the block was also treated. The insecticide successfully destroyed *Theraptus* adults and nymphs present on the spadices at the time of treatment but did not prevent a little damage from adults which, in spite of the guard row, migrated into the block during the periods between each dusting.

The crop of developing nuts on each palm at the beginning of the experiment comprised the yield from spadices produced during the previous 12 months, throughout which the palms were attacked by *Theraptus*. At the beginning of treatment the youngest spadix on each palm was labelled; the nut yield of this and subsequent spadices showed the effect of destroying *Theraptus* with γ BHC dust (fig. 1).

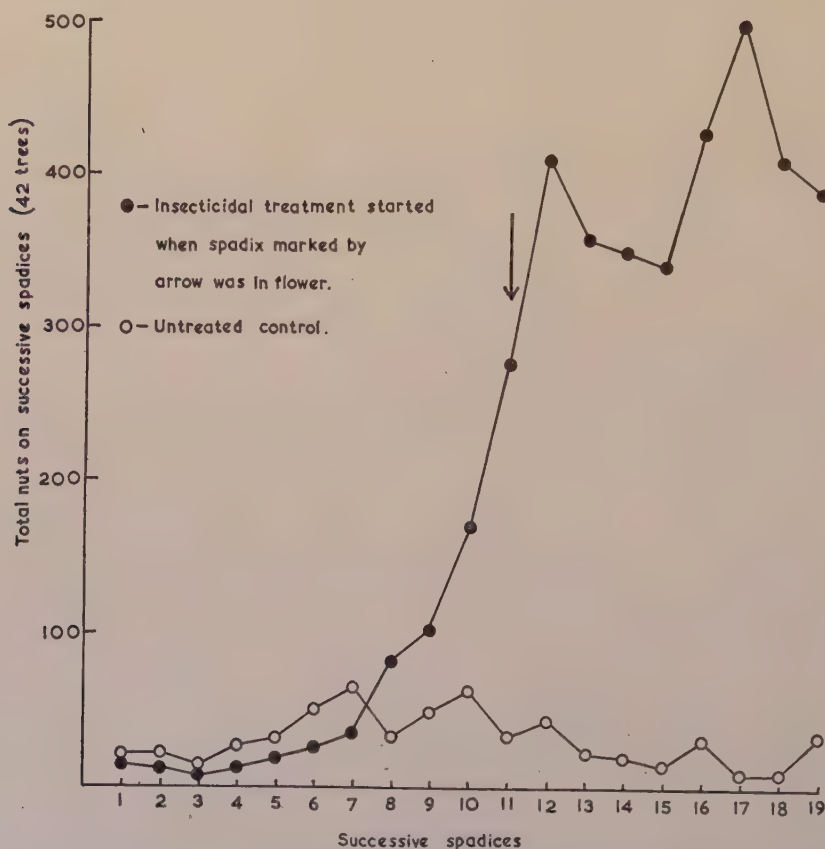


Fig. 1.—The effect of insecticidal control of *Theraptus* on the nut yield of a block of coconut palms at Mazizini, Zanzibar Island.

Fig. 1 shows that γ BHC, by destroying *Theraptus*, increased the nut yield from about 0.5 to 10 nuts per spadix. Some spadices, which had opened 1–2 months before treatment, also gave an increased nut yield; these bore a few

nuts which up to the time of treatment had escaped destruction but would ultimately have been destroyed but for the γ BHC treatment. The experiment shows that *Theraptus* may cause very serious crop loss, though in this plantation the conditions (a pure stand of good quality, closely planted palms, and absence of the predatory *O. longinoda*), greatly favoured the pest.

In this experiment the γ BHC-treated nuts became covered, when about 8 months old, by the Diaspidid *Hemiberlesia lataniae* (Sign.) (Pl. XI, fig. 6), which otherwise is not common. Presumably the insecticide or the diluent destroyed parasites or predators which normally control the scale insect.

Natural Nutfall in Relation to *Theraptus* Attack.

"Natural" nutfall occurs among 5-10-week-old nuts whereas *Theraptus* damage may cause fall of nuts up to about 16 weeks old.

The extent of "natural" nutfall and of natural plus *Theraptus*-induced nutfall was determined in an area of 101 palms at Kidichi, Zanzibar Island. Forty-four palms, colonised by *O. longinoda*, showed negligible *Theraptus* damage

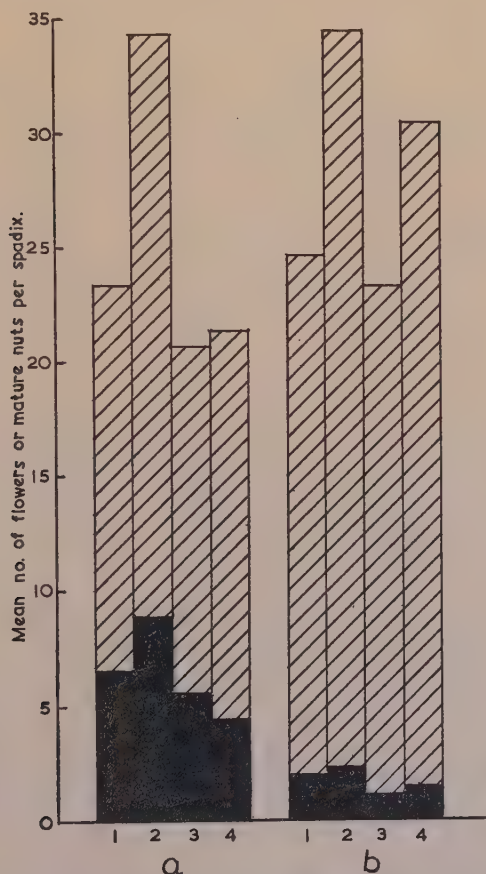


Fig. 2.—Effect of *Theraptus* attack on the mean numbers of female flowers and mature coconuts produced per spadix at different times of the year; Kidichi, Zanzibar Island. (a) undamaged palms, (b) palms damaged by *Theraptus*. 1 = spadices maturing Dec. 1950–Feb. 1951; 2 = March–June 1951; 3 = July–Sept. 1951; 4 = Oct.–Dec. 1951. Cross hatched blocks = mean no. of ♀ flowers per spadix. Solid blocks = mean no. of mature nuts per spadix.

and were used for determining "natural" nutfall. The remaining 57 were severely damaged and were used for determining "natural" plus *Theraptus*-induced nutfall. All spadices maturing during the periods, (1) Dec. 1950-Feb. 1951; (2) March-June, 1951; (3) July-Sept., 1951; (4) Oct.-Dec., 1951, were examined and fig. 2 shows the mean numbers of female flowers and mature nuts produced per spadix during each period. (The female flowers open about 12 months before the nuts become mature.)

It can be seen that undamaged palms (fig. 2a) produced more female flowers per spadix during the first than during the last half of the year. During the year the average "natural" nutfall was 74.8% compared with 94.0% of "natural" plus *Theraptus*-induced nutfall on palms which were exposed to *Theraptus* attack. More flowers were produced per spadix on the *Theraptus*-damaged palms than on the undamaged palms. Perhaps this is the normal response of a plant whose crop of developing fruit has been destroyed. These data show that *Theraptus* attack, prior to the time of natural nutfall, will destroy some nuts that would have abscised naturally. However, the few nuts that remain after natural nutfall has ceased, are still susceptible to damage by *Theraptus* (Table III).

TABLE III.

Percentages of female flowers and young nuts of the coconut palm lost from natural and *Theraptus*-induced nutfall (144 spadices maturing between 14.8.51 and 26.10.51 in block of 42 palms at Mazizini, Zanzibar Island).

Mean no. fls. per spadix	% destroyed as fls. by <i>Theraptus</i>	% natural and <i>Theraptus</i> -induced nutfall				% becoming mature nuts
		5-10 weeks old		10-14 weeks old		
		Undamaged	Damaged by <i>Theraptus</i>	Undamaged	Damaged by <i>Theraptus</i>	
35	49.1	16.5	27.4	0.2	4.4	2.2

Table III shows that, in one block of severely damaged palms, *Theraptus* destroyed an average of 49% of the unopened or newly opened flowers. Of the remaining 51% which developed further, 44% were lost within 10 weeks from natural and *Theraptus*-induced nutfall. After 10 weeks when natural nutfall had ceased 6.6% remained, but of those 4.4% were destroyed by *Theraptus* in the next 4 weeks, only 2.2% reaching maturity.

As has already been stated, palms differ in the number of female flowers they produce per spadix. Using the data obtained in the experiment at Kidichi (fig. 2) the palms were divided into groups according to the average number of flowers produced on their spadices, as follows:— (1) 1-10; (2) 11-20; (3) 21-40; (4) 41-60; (5) 61-80. The mean numbers of flowers and mature nuts produced per spadix were determined for each group, and the results obtained from the undamaged and from the damaged palms are shown separately in fig. 3.

It can be seen that for undamaged palms the number of flowers and number of nuts per spadix were positively correlated although, with increase in number of flowers, the ratio of nuts to flowers decreased. Thus, natural nutfall increased slightly with increase in the number of flowers produced per spadix. By contrast, on the *Theraptus*-damaged palms the nut yield, in relation to increase in number of female flowers, at first remained more or less constant and then decreased. The greater severity of *Theraptus* damage to palms bearing many flowers per spadix was noticed both in Tanganyika and in Zanzibar. Thus, from each of twelve palms at Mazizini, Zanzibar Island, eleven successive spadices each

bearing over 50 female flowers were examined; 111 of the 122 spadices bore a total of 8658 female flowers all of which had aborted due to *Theraptus* attack. The remaining 11 spadices produced 16 mature nuts, an overall average of 0.14 nuts per spadix. Six of the palms were then dusted heavily with 0.4% γ BHC

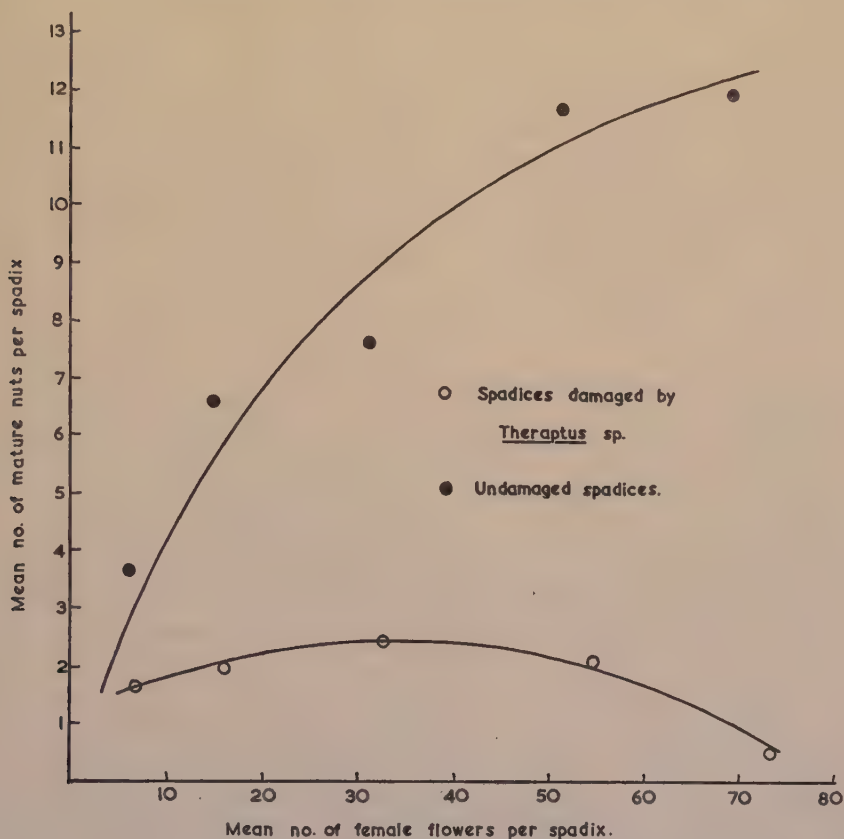


Fig. 3.—Effect of *Theraptus* attack on nut yield of coconut palms bearing different numbers of female flowers per spadix; Kidichi, Zanzibar Island.

once a week as previously described, while the remainder were untreated and acted as controls. Fig. 4 shows that the treatment increased the yield from about 0.14 to 17 nuts per spadix. The mean number of flowers per spadix being 78, it was calculated that for these palms, natural nutfall was about 78%, and natural plus *Theraptus*-induced nutfall, about 99.8%.

The food supply for *Theraptus* is greatest in palms which bear many flowers per spadix. Probably these can support a permanent population of the pest unlike those bearing few flowers, which occasionally escape re-infestation for sufficiently long to allow some nuts to reach a resistant age. This may be why *Theraptus* damage becomes more severe with increase in the number of female flowers.¹ Whatever the reason, it seems that damage could be reduced by selecting seed of palm varieties that bear relatively few female flowers per spadix. Such varieties are preferred in many countries because they yield a few large nuts requiring less work in collection and processing than the relatively large number of smaller nuts produced by palms which bear many flowers per spadix.

Alternative Host Plants of *Theraptus*.

A search for alternative host plants of *Theraptus* was not made, though in the Solomon Islands *A. cocophaga* has many hosts (Phillips, 1940), suggesting that the nearly related *Theraptus* is unlikely to be confined to the coconut palm.

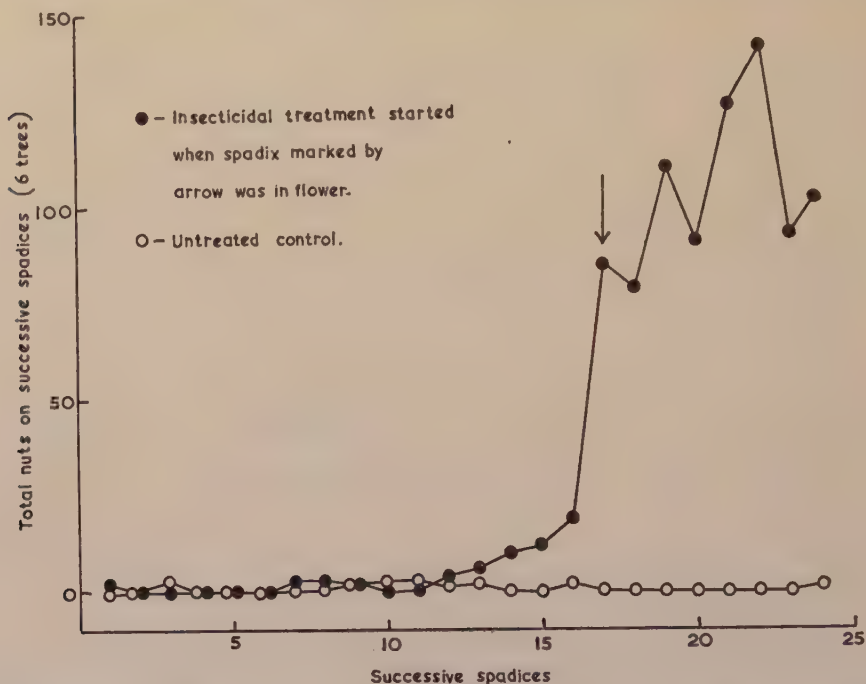


Fig. 4.—The effect of insecticidal control of *Theraptus* on the nut yield of coconut palms bearing many female flowers per spadix.

Following the author's recording of *Theraptus* on coconut palms in Pemba, Mr. G. D. Wilkinson, the Agricultural Officer for Pemba, found many nymphs and adults damaging developing fruits of cacao at Matangwatwani, Pemba. The author then found *Theraptus* nymphs on cacao in Zanzibar Island.

It has since been reported (Wright, 1952) that *Theraptus* has been found breeding on and damaging guava (*Psidium guajava*) and certain wild leguminous plants.

Summary.

Theraptus sp. is widespread in the coastal region of British East Africa where it severely damages developing coconut fruits.

The female may lay over 100 eggs. There are five nymphal instars and, in the field, it is probable that about nine generations are produced each year.

Damage to coconuts is similar to that caused by *Amblypelta cocophaga* China in the Solomon Islands. Female coconut flowers and young nuts may be destroyed by a single feeding puncture. Damaged 10–16-week-old nuts may reach maturity but are undersized and often distorted by lesions from which gummy material exudes.

Over 70% of 5–10-week-old nuts may fall "naturally"; thus *Theraptus* damages many nuts which would fall in any case. However, after natural nutfall

has ceased, the pest becomes concentrated on the few remaining nuts which are susceptible to damage for about four weeks more. One *Theraptus* may make over 200 feeding punctures in its lifetime. Consequently, a population density of less than two per palm may cause severe damage.

Palms bearing many female flowers per spadix suffer more severely from attack than those bearing few, although normally they give higher nut yields.

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I wish to thank Dr. J. Nutman, O.B.E., for his stimulating encouragement and criticism. I am indebted to the Director of Agriculture, Zanzibar, for the use of Government plantations and for the valuable co-operation of his staff. Grateful thanks are due to Miss E. M. Tait for the life history studies, and to Mr. Said Himid for assistance in field experiments. I also wish to acknowledge the co-operation of Dr. R. H. Le Pelley, Mr. G. Swaine, and Mr. H. J. Stanley in surveys made in Kenya and Tanganyika.

References.

- McDONALD, J. (1925). Annual report of the Mycologist for the year 1924.—Rep. Dep. Agric. Kenya, pp. 106–111.
- O'CONNOR, B. A. (1950). Premature nutfall of coconuts in the British Solomon Islands Protectorate.—Agric. J. Fiji, **21**, pp. 21–42.
- PHILLIPS, J. S. (1940). Immature nutfall of coconuts in the Solomon Islands.—Bull. ent. Res., **31**, pp. 295–316.
- WAY, M. J. (1951). An insect pest of coconuts and its relationship to certain ant species.—Nature, Lond., **168**, p. 302.
- WAY, M. J. (1953). The relationship between certain ant species with particular reference to biological control of the Coreid, *Theraptus* sp.—Bull. ent. Res., **44**, pp. 669–691.
- WELSFORD, E. J. (1925). Report of the Mycologist for the year 1924.—Rep. Dep. Agric. Zanzibar, 1924, p. 15.
- WELSFORD, E. J. (1926). Gummosis in coconuts.—Tech. Conf. E. Afr. Depend., Nairobi, pp. 205–210.
- WRIGHT, N. C. (1952). 7th Annual Report of the Committee for Colonial Agricultural, Animal Health and Forestry Research.—Colon. Res. Rep. 1951–52, p. 184.
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FIG. 1. Newly opened spadix of the coconut palm showing unopened male and female flowers.



FIG. 2. Female flower and 6—10-week-old nuts with bracts partly removed to show *Theraptus* damage.

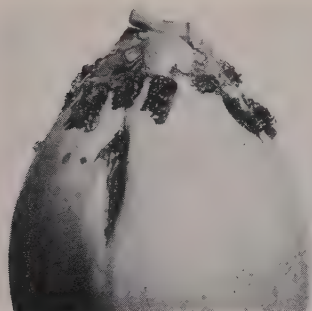


FIG. 3. 16—20-week-old coconut damaged by *Theraptus* about 2 weeks previously; gum is oozing from necroses which have appeared from beneath the bracts.

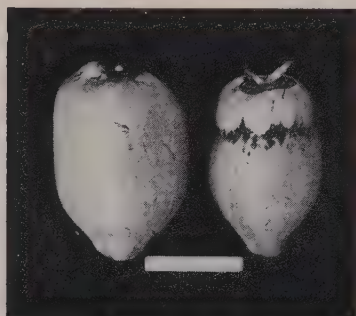


FIG. 4. Mature nuts from the same spadix: on the left damaged by *Theraptus* at about 5 months old; on the right damaged at about 4 months.

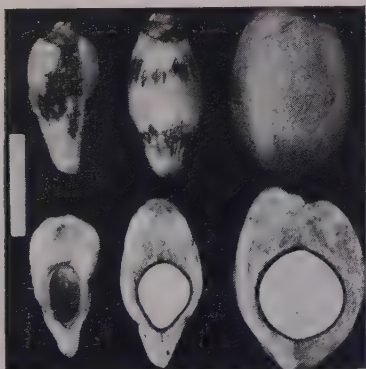


FIG. 5. Surface and section of mature coconuts showing effect of *Theraptus* damage on nut size. Left—endosperm destroyed by splitting of exocarp and endocarp; centre—undersized nut damaged at two periods during development; right—undamaged nut.



FIG. 6. Nine-month-old nuts from a palm treated each fortnight with 0.4% γ BHC dust. The Diaspidid, *Hemiberlesia lataniae* (Sign.) has been scraped away from the left side of one nut.

THE RELATIONSHIP BETWEEN CERTAIN ANT SPECIES WITH
PARTICULAR REFERENCE TO BIOLOGICAL CONTROL OF
THE COREID, *THERAPTUS* SP.

By M. J. WAY.

E.H.N.

Clove Research Scheme, Zanzibar.*

(PLATE XII.)

An undetermined species of the genus *Theraptus* (Coreidae), which severely damages developing coconut fruits in British East Africa, is controlled by the predatory ant *Oecophylla longinoda* (Latr.) (Way, 1951). This ant is locally abundant in coconut areas and, where it colonises at least 70% of the palms, damage by *Theraptus* is generally slight. In many areas, however, *O. longinoda* is uncommon, possibly because it suffers from competition with the ant *Pheidole punctulata* Mayr (Way, 1951). In others, it has been exterminated by either *Anoplolepis longipes* (Jerd.) (Nutman & Sheffield, 1949; Way, 1951) or *A. custodiens* (F. Sm.) which like *P. punctulata* do not protect coconut palms from damage by *Theraptus*.

Ant Species in Coconut Areas.

An incomplete list of the species of ants collected in coconut plantations in the Zanzibar Protectorate is given below:—

Dorylus (*Anomma*) *nigricans* Ill.

Polyrhachis (*Myrma*) *schistacea* (Gerst.) var. *rugulosa* Mayr

„ „ sp. (? race of *militaris* (F.))

Cerapachys sp.

Paltothyreus tarsatus (F.)

Anochetus sp.

Pheidole punctulata Mayr

Myrmicaria striata Stitz

Cardiocondyla wroughtoni (Forel) var. *bimaculata* Wh.

Crematogaster (*Acrocoelia*) *castanea* F. Sm.

„ sp. nr. *rectinota* (Forel)

Atopomyrmex mocquersyi André var. *curvispina* Forel

Meranoplus sp. nr. *nanus* André

Tetramorium gladstonei Forel

Triglyphothrix constanciae (Arnold)

Tapinoma luteum (Emery)

„ *melanocephalum* (F.)

Semonius schultzei Forel

Anoplolepis custodiens (F. Sm.)

„ *longipes* (Jerd.)

Acantholepis egregia Forel

Paratrechina longicornis (Latr.)

Plagiolepis brunni Mayr

Oecophylla longinoda (Latr.)

* Seconded from Rothamsted Experimental Station, Harpenden, Herts.

Camponotus (*Myrmamblys*) *vividus* (F. Sm.)
 „ (*Myrmosericus*) *rufoglaucus* (Jerd.) ssp. *cinctellus* (Gerst.)
 „ „ „ (Jerd.) ssp. *vestitus* (F. Sm.)
 „ (*Tanaemyrmex*) *maculatus* (F.)
 „ (*Myrmamblys*) sp.
 „ (*Myrmotrema*) sp.

Although only *O. longinoda* habitually nests in trees, five primarily ground-nesting species were found nesting on the aerial parts of coconut palms, usually in the spadices or leaf axils.

Most of the ant species form small, often widely scattered colonies and their influence appears insignificant, except perhaps close to their nests. *Myrmecaria*

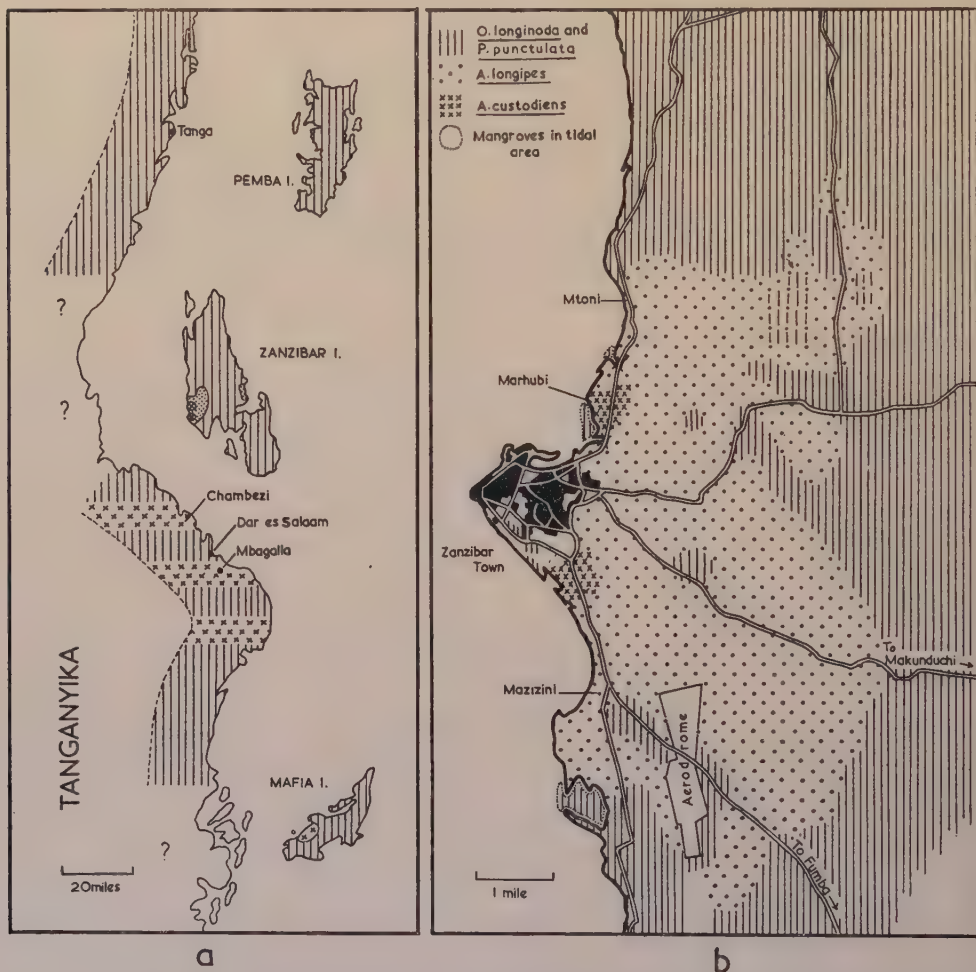


Fig. 1.—Maps showing present knowledge of the distribution of various ant species in part of Tanganyika and in the Zanzibar Protectorate.

a. Provisional map of whole area. In the Dar-es-Salaam area the alternation of crosses with vertical lines merely indicates that areas occupied by *A. custodiens* alternate with areas occupied by *Pheidole punctulata* and *O. longinoda* and is not representative of actual distribution.

b. Plan made during the year 1951 of the neighbourhood of Zanzibar town showing details of distribution of *A. custodiens* and *A. longipes*.

striata was occasionally found as single large colonies nesting in the soil but it forages in only a few trees, usually not coconut palms. This ant does not protect coconuts from *Theraptus* attack and its workers are preyed upon by *O. longinoda*.

Many ant species are predators while others may attend and greatly benefit various plant feeding Homoptera. Therefore it would appear that the ant species which are abundant will strongly influence general insect populations. In Zanzibar, they include the migratory *Dorylus* (*Anomma*) *nigricans*, which is well known as a predator, but is mainly a ground forager and was found only once on a young coconut palm. This ant does not protect coconuts from *Theraptus* even during the short periods when it is in a particular area and it does not attack *O. longinoda*. On the contrary, it is preyed upon by the latter (Way, in press). *D. nigricans* and other DORYLINAE are absent in areas densely populated by the ants *Anoplolepis longipes* and *A. custodiens* because in Zanzibar their colonies, like those of *O. longinoda*, are annihilated or driven off by these species. Consequently, *D. nigricans*, like *M. striata*, has little effect on the abundance of *Theraptus*.

Fig. 1 shows the distribution on the coast of East Africa of the four important ant species in 1951. *O. longinoda* and *P. punctulata* were widely distributed; *A. longipes* was recorded only in Zanzibar Island; *A. custodiens* was abundant in two small areas in Zanzibar Island and on parts of the neighbouring Tanganyika coast, and was also present in Mafia Island. *A. longipes*, *A. custodiens*, and DORYLINAE were absent in Pemba Island.

Biology and Habits of the Economically Important Ant Species.

Work on the biology of *O. longinoda* is reported elsewhere (Way, in press), and this paper deals with other species of ants concerned in *Theraptus* control.

Pheidole punctulata.

Some species of *Pheidole* are pests because they tend and thereby benefit species of PSEUDOCOCCIDAE which may either cause severe mechanical damage to plants (Kirkpatrick, 1927) or be vectors of plant virus diseases (Carter, 1933; Strickland, 1951). In the Solomon Islands, *P. megacephala* is destructive to a beneficial species of *Oecophylla* (Phillips, 1940; O'Connor, 1950).

In the coastal region of East Africa, *P. punctulata*, was abundant in all the coconut areas examined; near Mombasa in Kenya, near Tanga, Dar-es-Salaam, and on Mafia Island in Tanganyika, and on the islands of Zanzibar and Pemba in the Zanzibar Protectorate. It was unusually abundant in parts of Pemba Island.

The favourite nesting site of this ant is at the base of the trunk of the coconut palm beneath the bark which forms an "apron" over the junction of the surface roots with the bole of the tree (cf. O'Connor, 1950). When the apron is well developed, the nests are almost invariably found here. *P. punctulata* also nests at ground level inside rotting coconut husks and beneath dead vegetation. Nests in the soil did not extend more than eight inches below the surface and seemed to be built mainly when alternative sites were not available.

P. punctulata also nests in the crown of the coconut palm using the space between the inner and outer spathes (fig. 2), in which numerous workers, soldiers, winged sexual forms and brood were found. The arboreal nests are probably subsidiary to those in the ground because, (a) they were not found except in association with heavily populated nests at the base of the palm, the two being connected by ant runways, (b) ground nests were often the only type found, (c) gravid queens were not found in the arboreal nests. It is not known whether arboreal colonies of *P. punctulata* can thrive if isolated from the ground—an important point when control measures are considered.

In clean coconut plantations the nests of *P. punctulata* were generally found close to the palms, and it seemed that a separate ant colony was associated with each palm. Where a rich ground vegetation and undergrowth was present, the nests often occurred near various plants on which the ants tended honey-dew-producing Homoptera, including *Coccus viridis* (Green), two species of APHIDAE, and at least two species of PSEUDOCOCCIDAE.

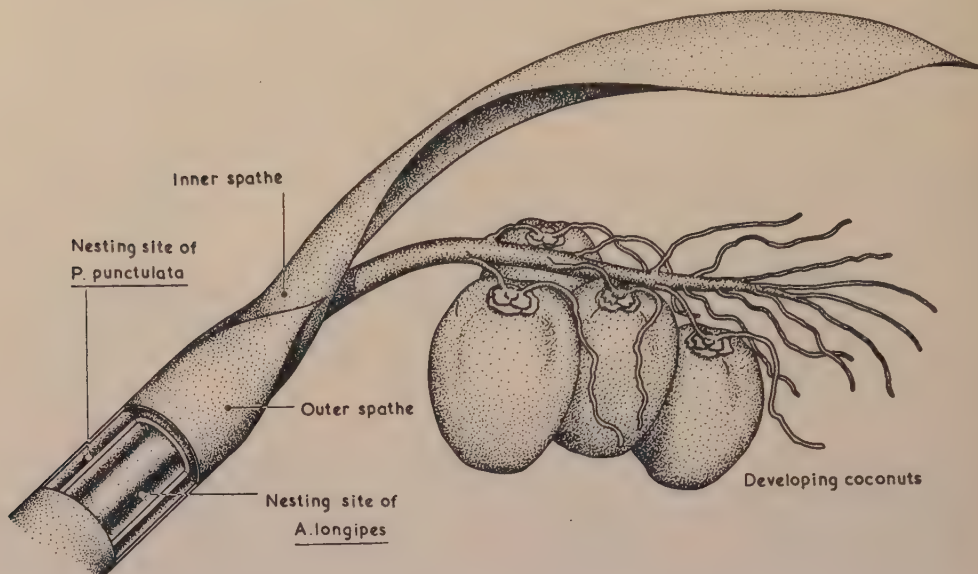


Fig. 2.—Diagram showing nesting sites of *Anoplolepis longipes* and *Pheidole punctulata* in the coconut palm spathe.

Runways from the ground nests to the crown were sometimes covered with an earthen roof, at any rate up the lower part of the trunk of the palm. Many covered runways were built during the wet seasons when it seemed that the exposed trunk was difficult to climb.

The main source of food for *P. punctulata* on the coconut palm is honey-dew from *Dysmicoccus* sp. ? *brevipes* (Ckll.) (on roots within the nests) and from *Pseudococcus citriculus* Green and *Cerataphis lataniae* (Boisd.) (on leaves and spadices in the crown). Newly open spadices were often covered with *P. punctulata* workers collecting nectar and pollen. The ants prevent *Theraptus* attack at this stage presumably by accidentally disturbing the pest. After about four weeks, however, when flowering ceases, few *P. punctulata* continue foraging on the spadix and *Theraptus* may then seriously damage the young nuts (Table II).

Anoplolepis longipes.

In Zanzibar Island, this species occupies two well-defined areas (fig. 1). The main area, in the neighbourhood of Zanzibar town, is extensively planted with coconut palms; the other contains few palms and consists of a strip of coastline about 300 yards wide and six miles long.

In the main area, *A. longipes* is generally abundant, in many plantations nests occurring under practically every palm. These are built in the soil to a depth of about 1.5 ft. and typically, each has a large main entrance up to about

8 ins. in diameter (Pl. XII, fig. 2), with several subsidiary entrances usually beneath a cover of litter. Inside the nest there is a large central chamber, the walls of which are supported by the living roots of the palm (Pl. XII, fig. 1). Brood is kept in pockets in its walls and in galleries leading from it, usually along plant roots.

Nests are usually built about six to twelve feet from the base of the trunk, and were rarely found under the "apron" which is the favourite nesting site for *P. punctulata*. *A. longipes* may nest in the crown of the coconut palm, either in rotting plant debris in the leaf axils or within the inner spathe of old or dead spadices (fig. 2). Again, the site differs from that chosen by *P. megacephala*, which uses the space between the inner and outer spathe.

Arboreal nests were frequently found; in a block of 127 coconut palms at Mazizini, ground nests were present at the base of every palm and 93 contained arboreal nests. Whether these are subsidiary to the ground nests or not has a bearing on the problem of control. Although arboreal nests sometimes contained brood, gravid queens were not found in them; also, they were always associated with nests at the base of the palm. In one experiment the trunks of seven palms containing arboreal nests were banded with grease to prevent movement to and from the ground; after eight months all *A. longipes* in the crowns were dead.

Where *A. longipes* was not common it sometimes formed colonies, each associated with up to 8 palms, but where the ant was abundant separate colonies could not be defined (see fig. 6). Colonies associated with isolated mango trees (*Mangifera indica*) were distinguishable, for around each tree were groups of nests from which streams of workers converged on the trunk.

In the coconut palm the main source of food is honey-dew from the Aphid *Cerataphis lataniae* which is often abundant on the young spadix, particularly on the under surface of the young inner spathe (fig. 2), and on the spike of unexpanded young leaves in the centre of the crown. *A. longipes* also solicits *Pseudococcus citriculus* and *Phenacoccus iceryoides* Green on the palm leaves. *Saissetia* spp. associated with *O. longinoda* on the coconut palm (Way, in press) were not found in trees occupied by *A. longipes*.

Numerous *A. longipes* were often associated with *Phenacoccus iceryoides* on mango trees. Sometimes *iceryoides* was very abundant on leaves, young stems and developing fruits which were thickly covered with a black growth of sooty mould fungi developing on the honey-dew.

Anoplolepis custodiens.

The areas occupied by *A. custodiens* (fig. 1) are well defined and are extensively planted with coconut palms and other trees.

The ground nests of this species differ from those of *A. longipes*. Each consists of one or more almost vertical shafts up to six feet long and 0.6–0.9 cm. in diameter which at intervals widen into brood chambers. Up to six shafts may be connected by horizontal galleries in the top six inches of soil (Pl. XII, fig. 3). The entrance to the nest is an approximately circular hole 1.4–2 cms. in diameter (Pl. XII, fig. 4). Unfortunately the crowns of palms were not examined for arboreal nests.

In densely populated areas the boundaries between ant colonies are not distinct (cf. *A. longipes*). Numerous *A. custodiens* are often associated with mango trees and a plan of part of the nest area of a community around an isolated tree is shown in fig. 3. Six main runways 27 to 53 yards long radiated from the tree across open ground on which were counted more than 1,300 nest entrances. An average of 1,180 worker ants per minute ascended the tree during a 1½ hours period (9.30–11 a.m. 4.x.51). It seems that single

colonies of *A. custodiens*, like those of *A. longipes*, are very large; alternatively several may be intimately associated.

In Zanzibar, it was noted that *A. custodiens* may solicit *Cerataphis lataniae* and a Pseudococcid species on the coconut palm, and *Phenacoccus iceryoides*

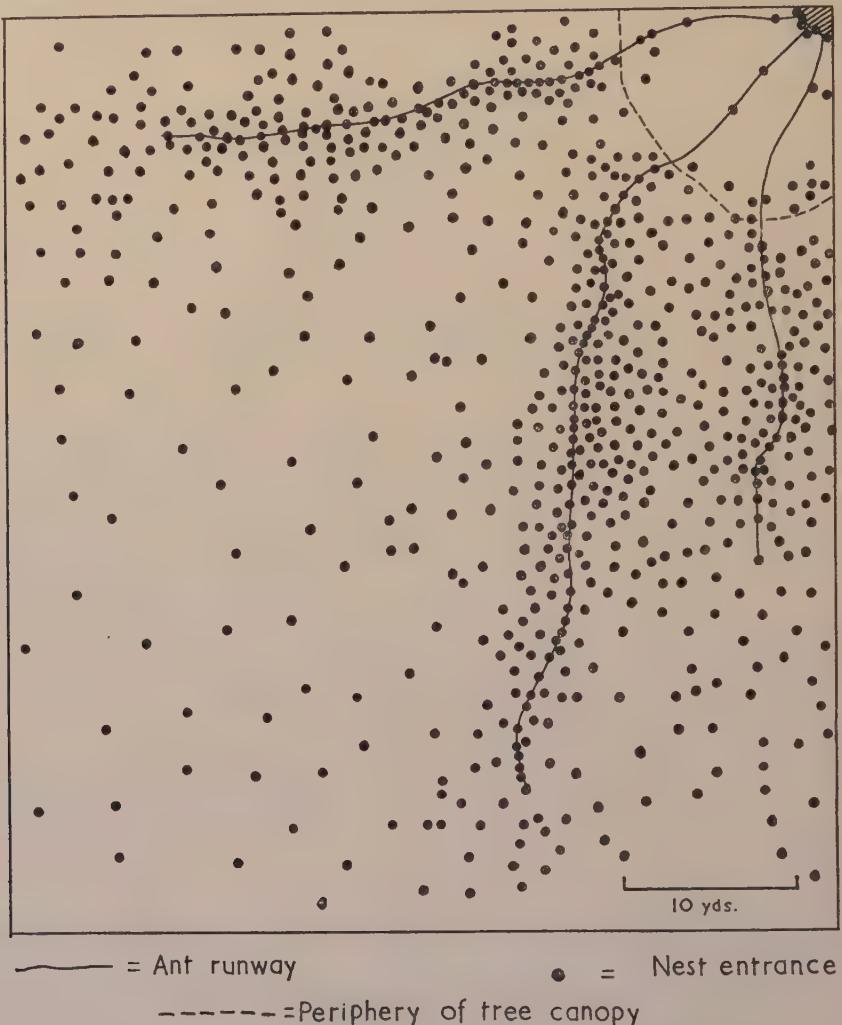


Fig. 3.—Plan of part of an area surrounding an isolated mango tree (*Mangifera indica*) showing three main runways used by *A. custodiens*, and the approximate number and position of nest entrances of the ant.

on the mango tree (cf. *A. longipes*). In Tanganyika, the Margarodid, *Aspidoproctus armatus* Newstead was solicited on *Cassia* sp. (Pl. XII, fig. 5). A careful search for the species of Homoptera tended by *A. custodiens* was not made.

Relationship Between Other Insect Species and the Dominant Ant.

O. longinoda is an efficient predator of many insect species and consequently few free-living insects are found in trees which it occupies (Way, in press). The

effects of *O. longinoda*, *A. longipes* and *P. punctulata* on other insects were compared. A coconut plantation was selected (Mazizini, Zanzibar Island) in which adjacent areas were dominated by the different ant species (as in fig. 6e). The crowns of ten palms occupied by each ant were treated with an insecticide—0.4% γ BHC dust (five palms) and a pyrethrin aerosol (five palms). The numbers of insects and spiders collected on 21-ft. square white sheets beneath each palm are given in Table I.*

TABLE I.

Insects and spiders collected from groups of five coconut palms occupied by three different ant species.

Insecticide	Dominant ant species	Other ants	Other insects	Spiders	Total
BHC	<i>O. longinoda</i>	3	22	15	40
	<i>A. longipes</i>	95	133	27	255
	<i>P. punctulata</i>	176	139	22	337
Pyrethrins	<i>O. longinoda</i>	0	32	12	44
	<i>A. longipes</i>	374	112	17	503
	<i>P. punctulata</i>	397	81	22	500

It can be seen that in palms occupied by *O. longinoda*, other medium-sized to large ant species were rare while several species, especially *Camponotus rufoglaucus* ssp. *cinctellus* and *Camponotus* (*Myrmotrema*) sp. were common in palms occupied by *A. longipes* and *P. punctulata*. Only one specimen of Coleoptera, and no Hemiptera-Heteroptera were collected from palms occupied by *O. longinoda*, while a total of seventy-six Coleoptera, including COCCINELLIDAE, and twenty-three Hemiptera-Heteroptera, including *Theraptus*, were collected from trees occupied by the other two ant species. Of fifty-seven insects collected from the palms occupied by *O. longinoda*, thirty were BLATTIDAE and twenty-three were Dermaptera. Although *O. longinoda* preys upon these, their presence is understandable because they occur among rotting plant debris in the axils of the leaves and spadices, an environment rarely foraged by the ant.

It will be clear from the above that the predatory *O. longinoda* kills many of the larger insects, while *A. longipes* and *P. punctulata* tolerate them.

Relationships of Ant Species to *Theraptus*.

Both nymphs and adults of *Theraptus* feed on the spadices of the coconut palm and thus are easily attacked by predators, or disturbed by foraging ants. The effects of *O. longinoda*, *A. longipes*, and *P. punctulata* on *Theraptus* were compared.

Effect of destruction of *O. longinoda*.

Twenty palms occupied by thriving *O. longinoda* colonies were selected. In ten palms the ants were destroyed using 0.4% dieldrin wettable powder sprayed around the base of the trunks and on the leaves. This treatment also kept the palms free from other species of ants for over eight weeks. At the time of treatment the most recently opened inflorescence in each palm was labelled, and fig. 4 shows the yields of nuts per ten palms on successive spadices ** before and after destruction of the ant.

* The figures do not include small insects or many winged species. Also, some insects, when affected by the insecticide, probably fell into the leaf axils and thus were not collected.

** On each palm a fresh spadix opens about once every 24 days throughout the year.

Spadices opening after the *O. longinoda* colonies were destroyed yielded virtually no nuts, due to attack by *Theraptus* which invaded the palms immediately the ant protection had ceased. The damage to spadices which opened about 24 and 48 days before the ants were destroyed shows clearly in the graph; young

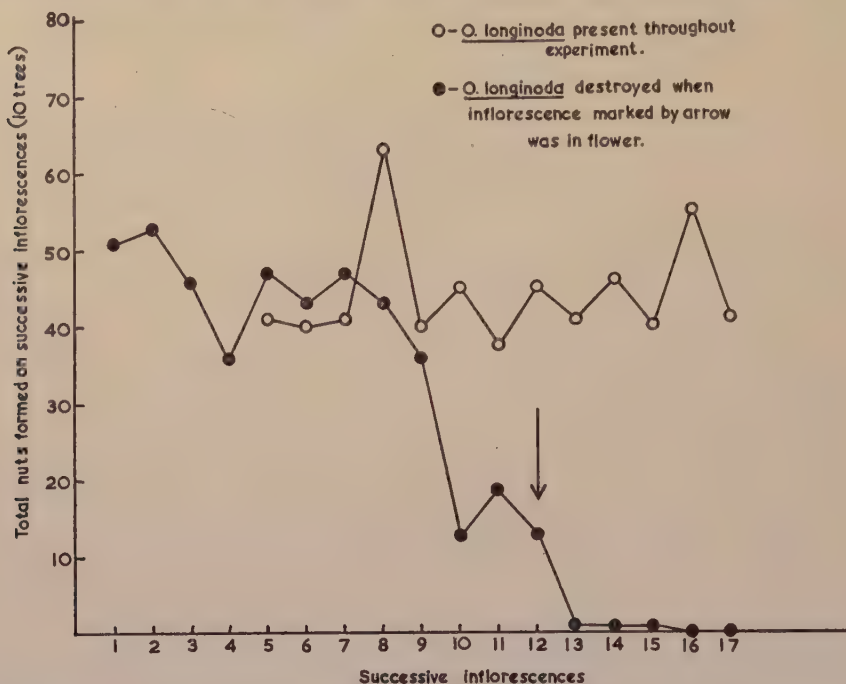


Fig. 4.—Effect of insecticidal destruction of *O. longinoda* on extent of damage caused to developing coconuts by *Theraptus*.

nuts are susceptible until about 4 months old; thus spadices nos. 10 and 11 in the graph (fig. 4), although undamaged at the time of treatment, were damaged later. Fig. 4 shows that high yields were maintained in the ten palms whose *O. longinoda* colonies were not destroyed.

Comparative effect of O. longinoda and A. longipes on Theraptus damage.

This experiment was carried out in a coconut plantation (Mazizini, Zanzibar Island) where *A. longipes* was destroying and replacing colonies of *O. longinoda* (see fig. 6). As before, nut-yields on successive spadices of ten palms were recorded before and after destruction of *O. longinoda* by *A. longipes*; yields were also recorded on ten neighbouring palms occupied continuously by *O. longinoda*. The curves for nut-yields (fig. 5) are essentially similar to those in fig. 4, although yields are somewhat higher, probably because the palms used in this experiment were more nearly mature. *A. longipes*, therefore, unlike *O. longinoda* does not prevent developing coconuts from being severely damaged by *Theraptus*.

Effect of A. custodiens on Theraptus damage.

Although no quantitative data were obtained, it was noticed that much *Theraptus* damage occurs on palms occupied by *A. custodiens*. At Mbagalla in Tanganyika (fig. 1) a plantation of very good palms, densely populated with *A. custodiens*, showed probably the most severe *Theraptus* damage observed in

East Africa. In August and November 1951, most trees in this plantation were almost devoid of mature and developing nuts and all the mature nuts examined were damaged, so it is clear that *A. custodiens*, like *A. longipes*, is not predatory on *Theraptus*.

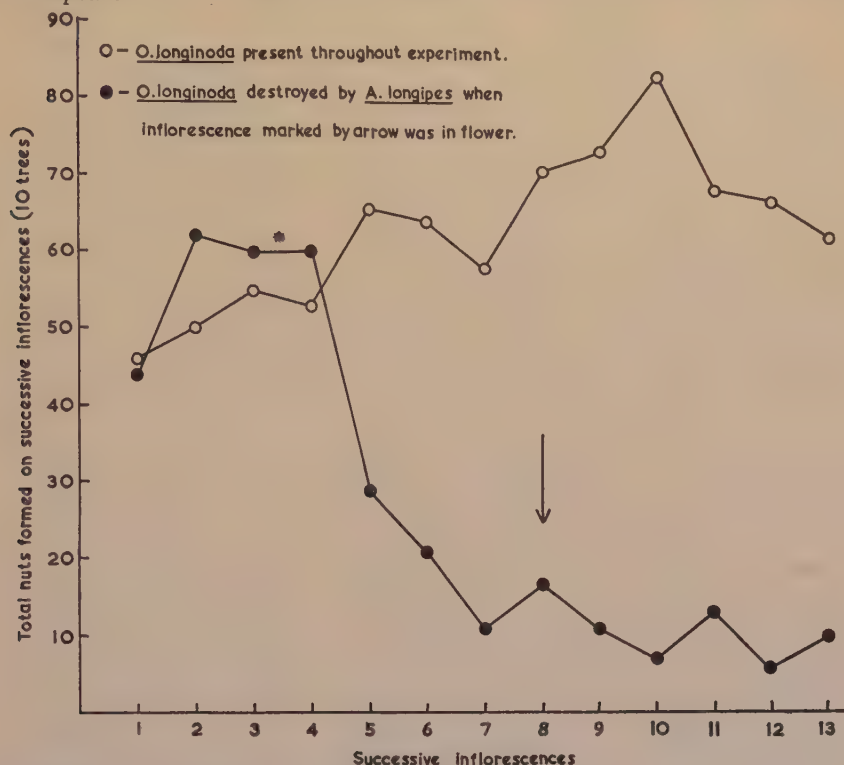


Fig. 5.—Effect of destruction of *O. longinoda* by *A. longipes* on the extent of damage caused to developing coconuts by the *Theraptus*.

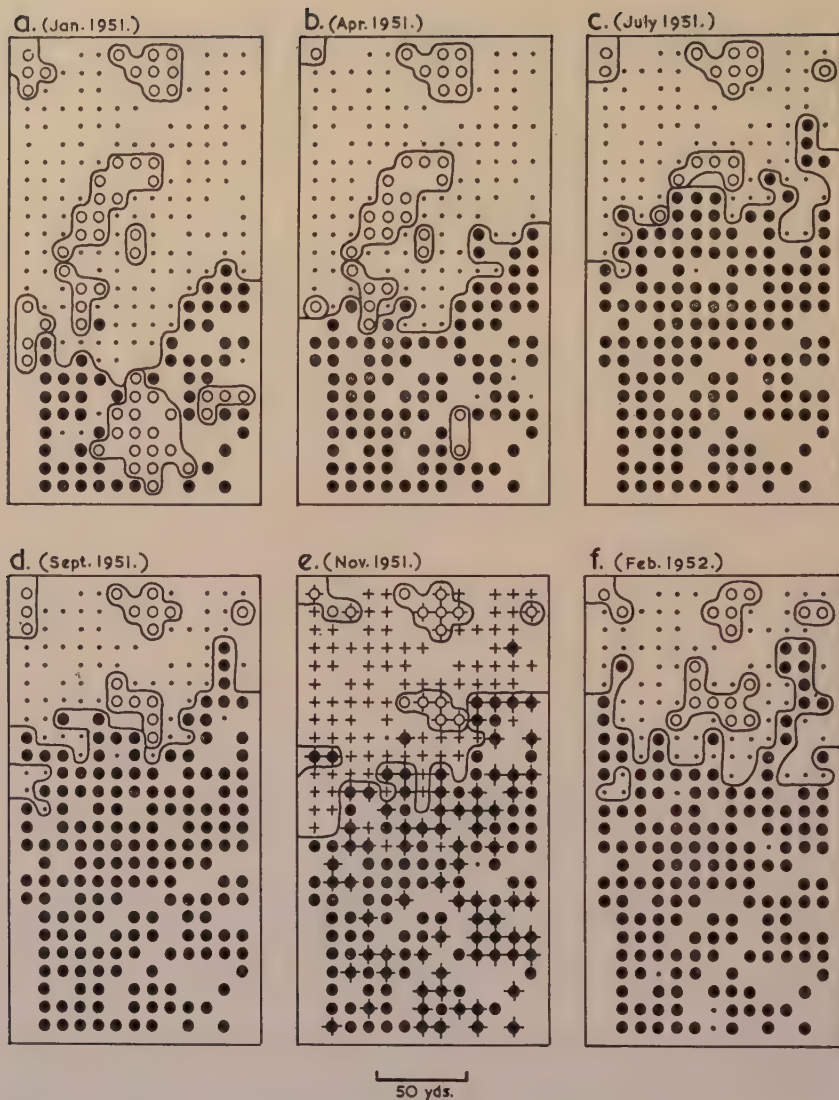
Comparison of the effects of O. longinoda and P. punctulata on Theraptus damage.

Outside the areas occupied by the two *Anoplolepis* species, most coconut palms are colonised either by *O. longinoda* or *P. punctulata* (see figs. 6e and 7). The distribution of the two species was determined in three blocks, each of about 1.5 acres, in a plantation at Kidichi, Zanzibar Island, and the nut-yield from each palm was recorded throughout 1951. Also a record was kept of nuts which

TABLE II.

Effects of *O. longinoda* and *P. punctulata* on *Theraptus* damage to developing coconuts.

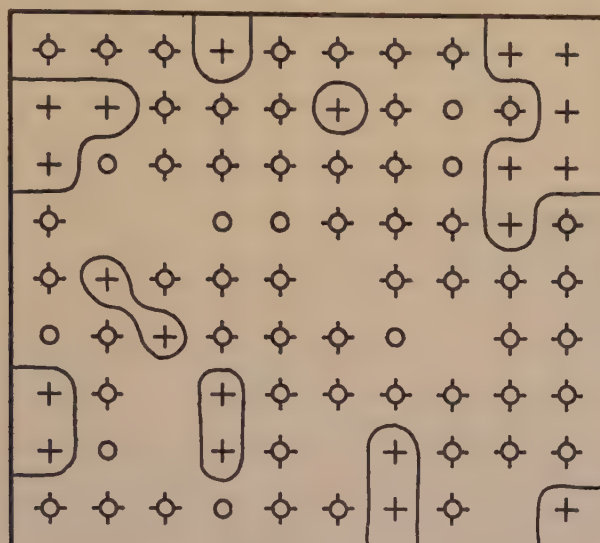
Dominant ant species	No. of palms	Nut yield per palm	% nuts showing <i>Theraptus</i> damage	
			<10 lesions	>10 lesions
<i>O. longinoda</i>	43	72.3	10.6	3.2
<i>P. punctulata</i>	115	13.4	28.9	62.2



- = Palm occupied by A. longipes
- = Palm occupied by O. longinoda
- * = Palm in which A. longipes and O. longinoda were absent.
- Approx boundary of territories occupied by A. longipes and O. longinoda.
- + Fig. 6e. P. punctulata nesting at base of palm.
- ◆ Fig. 6e. P. punctulata and A. longipes present on palm.
- ⊕ Fig. 6e. P. punctulata and O. longinoda present on palm.

Figs. 6a-f.—Plan of part of a coconut plantation at Mazizini, Zanzibar Island, showing spread of A. longipes into an area occupied by O. longinoda and P. punctulata.

were damaged but not destroyed, these being grouped into those with more and those with less than about ten lesions caused by feeding punctures. The results are shown in Table II, the yield being expressed as the mean number of nuts produced per palm during the year.



20 yds.

○ = Palm crown colonised by *O. longinoda*

+ = Palm base colonised by *P. punctulata*

⊗ = Palm occupied by both species

— Approx. boundary of territory occupied by *O. longinoda*.

Fig. 7.—Plan of part of a coconut plantation at Kidichi, Zanzibar Island, showing extent of occupation of individual palms by *O. longinoda* and *P. punctulata*.

It can be seen that, due to *Theraptus* attack, the nut yield from palms occupied by *P. punctulata* was less than one fifth of that from palms occupied by *O. longinoda*, and the damage to mature nuts was also severe. Of the nuts from palms occupied by *O. longinoda* 13.8% were damaged, perhaps because some palms were occupied by young or declining ant colonies comprising too few individuals to protect them completely.

Competition between *Anoplolepis* spp. and *O. longinoda*.

The *Anoplolepis* species are exotic. *A. longipes* has been in Zanzibar for over fifty years (Nutman & Sheffield, 1949), but *A. custodiens* was introduced or has become abundant only in the last fifteen years. The spread of *A. longipes* was noticed by local plantation owners, and Nutman and Sheffield (1949) observed that it attacked and exterminated the indigenous *O. longinoda*.

Fig. 6a-f shows the advance of *A. longipes* through part of a coconut plantation during which several *O. longinoda* colonies were destroyed. When the advancing *A. longipes* approached a palm occupied by *O. longinoda*, many large

workers of the latter descended to just above soil level and formed a dense 1 to 2 ft. broad band around the trunk. They combined in hostile movements, such as quivering of the limbs and raising of the abdomen but, when *A. longipes* workers ascended the tree and seized them one by one, they struggled but otherwise did not actively resist attack, nor did other *O. longinoda* workers assist the individuals attacked. This continued for several hours until most of the *O. longinoda* workers on the trunk had been destroyed; the remainder then retreated and formed bands in front of their nests. They were followed and attacked by *A. longipes* workers who eventually entered the nests and destroyed or carried away the remaining adults and brood.

The relatively passive resistance of *O. longinoda* to attack by *A. longipes* is remarkable because it normally attacks and kills many other insects, including other species of ants which *A. longipes* tolerates. It is significant that the ants attacked by *A. longipes*—the DORYLINAE and *O. longinoda*, are noted for their hostility, whereas the species it tolerates are not hostile and do not hinder it. It seems that aggression by *A. longipes* is a reaction only to hostility or hindrance by other insects.

Competition between *A. custodiens* and *O. longinoda* was not seen because, in Zanzibar, the former (see fig. 1b) occurred only within areas occupied by *A. longipes*. In an *A. custodiens* area, a colony of *O. longinoda* was artificially established on a young mango tree, the trunk of which was grease-banded to exclude *A. custodiens*. The colony flourished, and, after five months, the grease band was bridged with strips of wood. *A. custodiens* invaded the tree within one hour and within eighteen hours had destroyed or carried off all the *O. longinoda* workers and brood, so this ant, like *A. longipes*, does not tolerate *O. longinoda*. This is confirmed by the absence of *O. longinoda* in areas occupied by *A. custodiens*.

Relationship of *P. punctulata* to *O. longinoda* and *A. longipes*.

The distribution of these three ant species was determined in an area, part of which is shown in fig. 6e. For *A. longipes* and *O. longinoda*, only their presence or absence in the crown of each palm was recorded, but, for *P. punctulata* the palms were separated into those in which (a) the ant was absent; (b) nests were present only at the base of the palm, the workers not foraging in the crown; (c) nests were at the base from which workers were ascending the palm and foraging in the crown; (d) nests were present both at the base and in the crown. Categories (b)–(d) indicate increasing abundance of *P. punctulata* in the palm.

TABLE III.

Status of *P. punctulata* colonies in coconut palms, some occupied by *A. longipes* and *O. longinoda*.

Details of ant species		% palms with <i>P. punctulata</i> absent	% palms with <i>P. punctulata</i> nesting at base		
Species	No. of palms occupied		Palm crown not foraged	Palm crown foraged	Nests present in crown
<i>A. longipes</i> ..	162	44	52	4	0
<i>O. longinoda</i> ..	53	19	68	7	6
Above spp. absent	83	5	9	35	51

The results in Table III show that *A. longipes* and, to a less extent, *O. longinoda* lowered the population of *P. punctulata*. In the crown of 13% of the palms occupied by *O. longinoda*, the ant was competing with *P. punctulata*, the two species occupying different territories. Here, *O. longinoda* workers were hostile to *P. punctulata* which invaded their territory. Worker *P. punctulata* were seen holding down an *O. longinoda* worker while their soldiers "jointed" its limbs and body with their mandibles. Also, living *O. longinoda* workers were seen with the severed heads and mandibles of *P. punctulata* workers still attached to their limbs and antennae; they had bitten off the thorax and abdomen of the attackers, and thus escaped.

P. punctulata was seen attacking an *O. longinoda* colony which had been artificially established on potted clove seedlings on a small table (Way, in press), the legs of which were placed in dishes of oil to stop entry of *P. punctulata* which was abundant in the area. In one experiment the dishes were bridged with strips of wood and within two days *P. punctulata* workers had entered the soil of some of the pots. At this stage, the *O. longinoda* workers showed hostility but were not seen attacking the invaders; they built an apparently protective web of silk between the stems and leaves of the seedlings several inches above the soil surface. Nevertheless, *P. punctulata* workers invaded the aerial parts of some seedlings and the *O. longinoda* retreated to others. One by one these were invaded and, after 23 days, all the workers and brood had been destroyed by *P. punctulata*, some of which were also killed.

P. punctulata must limit *O. longinoda* in the field although its effect is not so clear as that of the two *Anoplolepis* species. In the Solomon Islands, O'Connor (1950) suggested that *P. megacephala* colonies at the base of a palm trunk form a barrier across which *O. longinoda* workers are unable to pass to colonise other palms. In Zanzibar *P. punctulata* seems important mainly because it competes with, and destroys, *O. longinoda* colonies in the crown of the palm and also kills the newly established unprotected queens (Way, in press). This was observed in the nursery as well as in the field and as *P. punctulata* is abundant in the crowns of many palms, it must cause heavy loss of *O. longinoda* queens engaged in forming new colonies.

Competition between *A. custodiens* and *A. longipes*.

Some field observations were made on the competition between *A. custodiens* and *A. longipes*. In Zanzibar Island, *A. longipes* colonies were not found inside the two areas occupied by *A. custodiens*. At the boundary between the areas occupied by the two ants, workers of *A. longipes* were deterred when they encountered *A. custodiens* workers. During 1951, at Marhubi (fig. 1b), *A. custodiens* spread 50 to 100 yards into an area occupied by *A. longipes*, the latter being driven off or killed in the process. Thus *A. custodiens* is intolerant of *A. longipes* and, under certain conditions, may replace it, but in Zanzibar it occurs only within an area occupied by *A. longipes* and consequently there is no immediate danger that its spread will increase *Theraptus* damage. *A. custodiens* workers are, however, more aggressive than those of *A. longipes* and also have an unpleasant bite; they are troublesome to domestic animals which when tethered have been known to be killed.

Distribution of Ant Species in Relation to Soil and Vegetation.

There are distinctive soil types in Zanzibar Island which may greatly influence the natural vegetation and the crops grown (Calton, 1949; Tidbury & Calton, 1950). Calton (1949) has classified these soils into three main groups:—

(a) *Kinongo soils* which overlie porous coralline limestone and show a maturity sequence from vestigial humic soils (Kinongo d) to deep loamy to heavy soils (Kinongo a).

(b) *Changa soils*. These are deep soils showing a sequence from almost pure sands (Changa e) to sandy to heavy soils (Changa a).

(c) *Namo soils*, which are sluggishly draining heavy clays.

Distribution of O. longinoda.

In Zanzibar, *O. longinoda* is absent in certain of the areas of vestigial soils (Kinongo d), which are devoid of the necessary tree or bush vegetation. The ant is most abundant in the areas of fertile Kinongo a and b and Changa a and b, soils which often support a rich tree, bush and ground vegetation. It can thrive, however, in areas showing a wide range of soil types from almost pure sands to heavy clays, including the tidal clays of mangrove swamps (fig. 1b). Consequently soil type becomes a limiting factor to an arboreal ant, such as *O. longinoda*, only when it cannot support suitable tree or bush vegetation.

Distribution of A. longipes.

Within the areas it occupies, *A. longipes* is abundant in the very sandy soils (Changa d and e) and is also common in Changa c and in the vestigial and shallow soils (Kinongo c and d). These comprise practically all of the areas at present occupied. Namu soils occur some miles from the nearest *A. longipes* area and it is not known whether the ant can colonise them.

To the east and south-east of the main occupied area there is, in places, a sharp boundary between sandy soils (Changa d and e) and loamy or heavy soils (Changa b, Kinongo b). This boundary corresponds with that of the *A. longipes* area. Soil samples were collected at 6 and 18 ins. depth inside and also outside the boundary and were mechanically analysed by the standard International Method (Table IV).

TABLE IV.

Percentages of the constituents of various soils in relation to distribution of *A. longipes*.

Area examined	a				b					
	(Mile 6 Makunduchi rd.)				(Mile 6 Fumba rd.)					
	Changa e		Changa b		Changa d		Kinongo c		Kinongo b	
Status of <i>A. longipes</i>	Present		Absent		Present		Variable		Absent	
Soil depth ..	6 in.	18 in.	6 in.	18 in.	6 in.	18 in.	6 in.	18 in.	6 in.	18 in.
Coarse sand ..	65.0	69.3	28.3	22.9	60.5	43.1	26.5	25.1	31.9	26.4
Fine sand ..	28.8	25.4	23.0	17.9	27.3	23.9	25.5	16.3	30.9	22.3
Silt ..	1.7	2.3	3.5	1.0	1.7	2.3	11.5	7.0	3.0	1.0
Clay ..	4.0	3.3	38.0	55.3	8.7	29.3	25.7	43.5	28.0	43.7

In the Makunduchi road area, *A. longipes* was abundant in the sandy soil (Changa e), while it was absent and *O. longinoda* was present in the adjacent area of relatively heavy soil (Changa b). In the Fumba road area, *A. longipes* was abundant in the sandy Changa d and present, together with *O. longinoda*, in parts of the Kinongo c. This is not a sandy soil (see Table IV) but, being well drained and less than 2 ft. deep, it tends to be semi-arid in the dry season.

A. longipes was absent and *O. longinoda* was present in the Kinongo b soil which is over 4 ft. deep and supports a richer vegetation than Kinongo c.

Although *A. longipes* is mainly present in sandy and in shallow soils, and is generally absent from the deep loamy or heavy soils, it seems that soil type is not the only factor affecting distribution of the ant. For example, *A. longipes* is absent in the sandy soils which extend for at least seven miles north from the main area occupied. Most of the main *A. longipes* area lies within about seven miles of Zanzibar town (see fig. 1b) and is overcultivated or overgrazed; as a result ground vegetation is sparse, grasses in many places having been largely replaced by weeds. On the other hand, the sandy soils beyond about 6 miles north of Zanzibar town are not heavily grazed and cultivated and generally support a rich ground vegetation of grasses and creepers. Here *A. longipes* is absent and *O. longinoda* present. A difference in vegetation was also seen at the perimeter of the *A. longipes* area near the Makunduchi and Fumba roads where it seems that, outside the occupied area the more fertile soils, such as Changa b and Kinongo b, are intrinsically capable of supporting a richer ground vegetation than the sandy Changa d and the shallow Kinongo c, and also are less affected by cultivation and grazing. At three places on the boundary of the *A. longipes* area, the relatively sparse ground vegetation within it was dominated by pest weeds (*Oldenlandia bojeri*, *Stachytarpheta* spp., sometimes *Imperata cylindrica*, with *Lantana camara* locally abundant). A quarter to half a mile outside the boundary these species, although present, were rare, apart from *I. cylindrica* which was locally common, the dominant vegetation consisting of a thick growth mainly of grasses and creepers (e.g., *Panicum trichocladum*, *Paullinia pinnata* and *Commelina* spp.).

Thus it seems that the distribution of *A. longipes* may be influenced by the type of ground vegetation and perhaps only indirectly by the soil type though it is possible that the sandy soils are intrinsically more suitable than the heavier soils for *A. longipes*.

Although a rich vegetation benefits *O. longinoda*, it is clear that competition from this ant does not prevent the spread of *A. longipes* because where the former occurs it often occupies only a small part of the available terrain; also it was not seen repelling *A. longipes*. This suggests that thick vegetation itself is unsuitable for *A. longipes* possibly because it cuts out radiation from the sun and thus lowers the nest temperatures. This is shown in Table V which gives temperature records made in soil unshaded by tree or bush vegetation during sunny periods between 2 and 3 p.m. on successive days (3-4.x.51).

TABLE V.

Soil temperatures (°C.) within and outside areas occupied by *A. longipes*.

Area examined			Mile 6, Makunduchi rd.		Mile 4-6, Mtoni	
Soil type	Changa e	Changa b	Changa d	Changa d
Ground vegetation	Sparse	Rich	Sparse	Thick
Status of <i>A. longipes</i>	Present	Absent	Present	Absent
Depth of soil thermometer	$\frac{1}{4}$ in.	45	31	58	32
" " " "	3 ins.	37	29	42	30
" " " "	6 ins.	33	28	35	28

Distribution of A. custodiens.

In Zanzibar Island, *A. custodiens* occupies two small areas of heavily grazed sandy soil (Changa d and e) and, in one area at least, is spreading into the surrounding Changa d and e soils, at present occupied by *A. longipes* (fig. 1b). The mechanical analysis of a Changa e soil densely populated by *A. custodiens* is given in Table VI. The soils profile is shown in Plate XII, fig. 3.

TABLE VI.

Percentages of various constituents of a soil colonised by *A. custodiens* at Marhubi, Zanzibar Island.

	6 in.	18 ins.	3 ft.	6 ft.
Coarse sand ..	76.8	73.7	70.0	60.0
Fine sand ..	19.9	22.8	26.1	36.0
Silt	1.3	1.5	1.7	2.0
Clay	1.7	2.0	2.0	2.3

It has been mentioned that roads to the north and south of Dar-es-Salaam, Tanganyika, pass through belts in which *A. custodiens* is alternately present and absent (fig. 1a). There is a striking relationship here between soil type and distribution of the ant. The soil in areas occupied by *A. custodiens* consists of loose sands, at any rate in the upper horizons, and in the dry season of August 1951, wheel tracks made deep ruts in the unmetalled roads. Where *A. custodiens* was absent the soil was also sandy but compacted more readily, giving a relatively firm road surface. The presence or absence of *A. custodiens* in an area could be predicted with accuracy from the nature of these tracks. Mechanical analyses of soils occurring within and outside four of the *A. custodiens* areas near Dar-es-Salaam are given in Table VII, and it can be seen that *A. custodiens* is associated with soil containing much coarse sand and little clay.

TABLE VII.

Percentages of various soil constituents in relation to distribution of *A. custodiens*.

Area examined	Near Chambezi				Near Mbagalla						Near Dovia		Near Kongowe	
Status of <i>A. custodiens</i>	Present		Absent		Present			Absent			Pre-sent	Ab-sent	Pre-sent	Ab-sent
Soil depth	6 ins.	18 ins.	6 ins.	18 ins.	6 ins.	18 ins.	3 ft.	6 ins.	18 ins.	3 ft.	6 ins.	6 ins.	6 ins.	6 ins.
Coarse sand	86.1	76.9	71.1	66.6	87.2	82.8	80.6	65.5	60.4	60.9	88.4	73.5	71.1	58.2
Fine sand	10.2	17.0	21.4	24.2	9.0	9.7	9.9	24.4	16.0	16.7	7.2	14.0	23.8	29.5
Silt	0.5	0.7	1.7	1.7	0.7	1.0	0.5	1.5	1.5	1.5	1.0	1.0	1.0	3.0
Clay	2.0	4.0	4.7	6.0	2.5	6.3	8.7	7.7	20.0	19.3	3.0	10.5	3.0	7.0

As with *A. longipes*, the distribution of *A. custodiens* was correlated with differences in ground vegetation, except in one area (Mbagalla), where at the time of examination (August 1951) the vegetation seemed to be as sparse in an unoccupied area as in a neighbouring area occupied by *A. custodiens*. In Tanganyika many *A. custodiens* were nesting in almost bare exposed soil (Pl. XII, fig. 4) just outside the boundary of a plantation of young trees (*Cassia* sp.). The main food supply of the ant was honey-dew from *Aspidoproctus armatus* (Pl. XII, fig. 5), which was present on the trunks of the trees, but no nests were found beneath the trees nor were any workers seen tending the Margarodid or foraging for more than 15-20 yards inside the plantation. Similarly at Marhubi, Zanzibar Island, numerous *A. custodiens* were nesting in open ground sparsely covered with vegetation which adjoined a block of closely planted mango trees. Almost the sole food supply of the ant was honey-dew from *Phenacoccus iceryoides* which was being solicited by worker ants on trees at the edge of the block. Towards the centre of the block, the ants were scarce or absent on the ground and absent in several trees, two of which were colonised by *O. longinoda*. Tree-shaded ground, therefore, is avoided as a nesting site for *A. custodiens*, and it might be expected that any vegetation which casts a heavy shade would also be unsuitable.

Fig. 3 gives the distribution of nests of *A. custodiens* around an isolated mango tree and it can be seen that few were built beneath the tree canopy, the great majority being in the very sandy soil (Table VI) of the surrounding sparsely covered open ground. Soil temperatures were recorded during sunny weather with occasional cloud at intervals for 38 hours (4.x.51 to 5.x.51) beneath the tree canopy, which excluded most sunlight, and also in the unshaded open ground 20 yards from the trunk. The results in fig. 8 show that in the open, considerable diurnal temperature fluctuation occurred near the soil surface, the temperature rising to almost 60°C. in the day and falling to about 25°C. at night. At 1 ft. the temperature remained constant at about 30°C. in the open and 25°C. in the shade.

Trenches were dug to a depth of 5 ft. (near the lowest limit reached by *A. custodiens* nests) both in tree shade and in the open. At 2, 3 and 5 ft., thermometers were inserted horizontally 1 ft. into the undisturbed wall of each trench, which was then filled in. Three months later (22.xii.51) when the trenches were reopened the temperature at 2, 3 and 5 ft. was 29.5°C. in the open and 26°C. in the shade. Thus, even at 5 ft. there was a difference of 3.5°C. between tree-shaded and unshaded ground.

It is significant that, in the unshaded ground, the nest of *A. custodiens* (Pl. XII, fig. 3) with its brood chambers at different depths from 3 ins. to nearly 6 ft. provides a range of temperatures for rearing the brood. Fig. 8 shows that, by choosing the appropriate chamber, the ant can maintain its brood at a temperature which does not fall below 30°C. at night and can be kept constant at any level from 30°C. to at least 35°C. for over eight hours on a sunny day. By contrast, the maximum nest temperature in the shade was 25°C. during the night and 26°C. for about five hours during the day. The optimum temperature for *A. custodiens* is not known, but, if it is in the region of 35°C., it is probable that the relatively low soil temperature under the shade of tree or thick ground vegetation limits the distribution of the ant.

Distribution of Pheidole punctulata.

In Zanzibar and Pemba Islands this ant is present and often common in both sandy and heavy soils, and is sometimes abundant in areas bearing a thick ground vegetation. In one area of 80 palms (at Selem, Zanzibar Island) grasses and creepers were knee-deep and yet colonies were present at the base of 94% of the palms. Thick ground vegetation was also present in the area shown in fig. 7

where 90% of the palms had nests at their bases. Although details of the relative status of *P. punctulata* in different soil and vegetation types were not obtained, it is evident that, unlike the *Anoplolepis* species, the distribution of this ant in Zanzibar is not markedly influenced by these factors.

The world distribution of *Pheidole* species compared with that of the *Anoplolepis* species (Wheeler & others, 1922) suggests that if soil temperature is a limiting factor the former can thrive at temperatures too low for the latter to exist. It

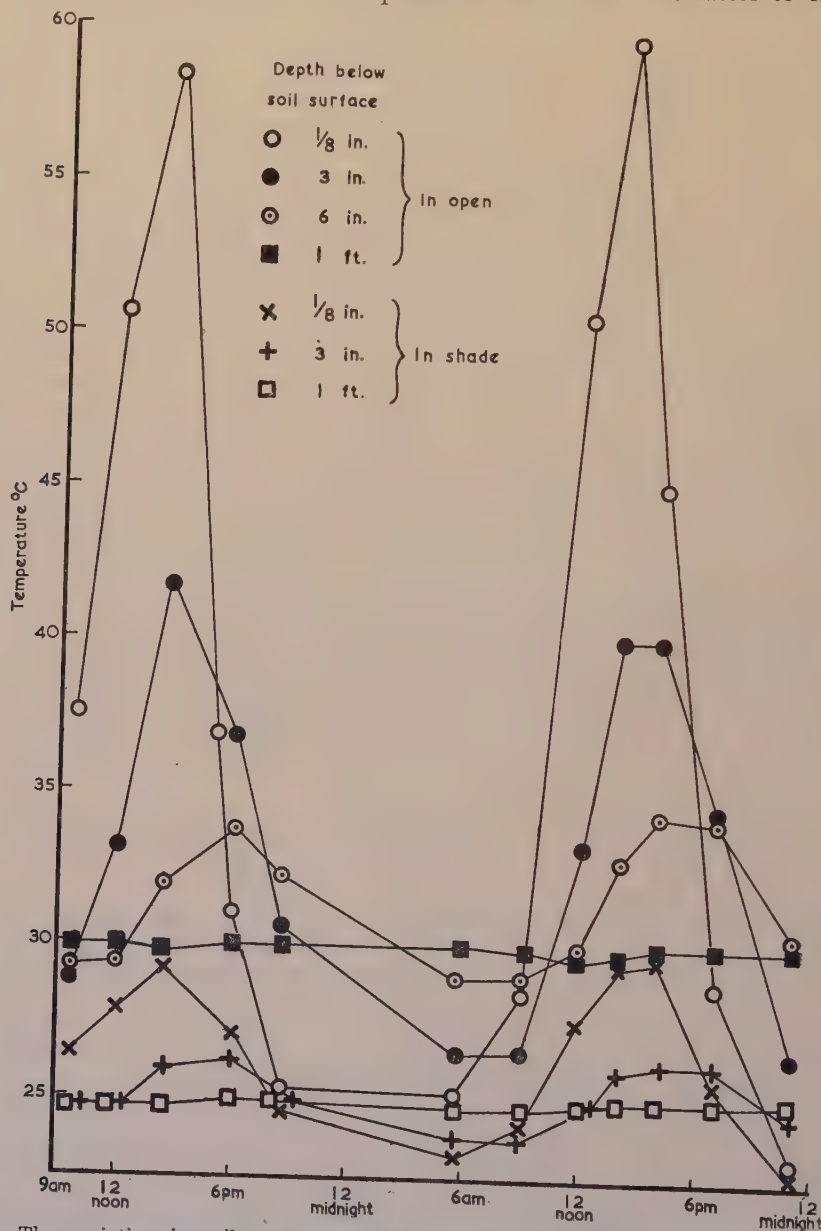


Fig. 8.—The variation in soil temperature at different depths during a period of two days (4th–5th Oct. 1951) in open ground and beneath the shade of a mango tree in an area (Marhubi, Zanzibar Island) colonised by *A. custodiens*.

is of interest that in the coffee-growing areas of the Kenya Highlands at over about 5,000 ft., *P. punctulata*, which normally nests beneath the coffee bushes, builds many nests during the wet seasons on the bare ground of the roads and tracks passing through the plantations. Perhaps this is associated with temperature, for, during the wet seasons, day as well as night temperatures are relatively low at this altitude, and nests on bare ground will receive most benefit from the available insolation.

Control of Undesirable Ant Species.

The present work shows that the harmful ant species must be controlled or suppressed before *O. longinoda* can be successfully used to control *Theraptus*. It seems that *A. custodiens* and *A. longipes* could be controlled by encouraging the growth of a thick ground vegetation of grasses and creepers. This would involve limitation of grazing and cultivation, and perhaps artificial establishment of plants which give heavy ground cover. This may be impracticable in many areas, but it should be emphasised that an extension of overgrazing and over-cultivation may lead to the further spread of *Anoplolepis*, and consequently to destruction of *O. longinoda* and increase in the damage caused by *Theraptus*.

Where cultural control is impracticable, there remains the possibility of control by insecticides. The fact that colonies of the two *Anoplolepis* species radiate from trees which, at any rate in clean plantations provide practically all their food, suggests that treatment of the tree trunks with a persistent type of insecticide is a possible method of destroying the ant. This treatment has advantages over treatment of the nest area for (a) the area to be treated is small, (b) the treated surface (bark of the tree) is relatively non-absorbent compared with soil and ground vegetation, and (c) the insecticide film is deposited on a vertical surface and is often shaded by foliage and thus likely to be more persistent than a film on the horizontal soil surface which receives the direct rays of the sun and may reach a temperature of over 60°C. (fig. 8).

A preliminary trial was carried out in an area (Marhubi, Zanzibar Island) heavily populated with *A. custodiens*. The following spray treatments were each applied to groups of 3 large mango trees (circumference of trunk 11–20 ft.) foraged by *A. custodiens*: (a) 2% technical dieldrin (85% active principle plus 15% other toxic materials), (b) 2% pure DDT and (c) 2% pure γ BHC. Each insecticide was emulsified in a medium of rain water containing 0.2% w/v. cyclohexylamine dodecyl sulphate and 5% v/v. benzene. A control group of three similar mango trees was treated with the medium alone. The treatment consisted of spraying a band about 3 ft. wide around each tree trunk near its base, the volume used per tree varying from about 800 to 1,500 cc. according to its diameter.

The effect of the insecticides was partly assessed by counting the ants ascending the tree trunks at intervals after treatment. The circumference of the trunk was divided into 6 in. sections, and the number of ants passing up each section was counted over a period of one minute. In this way an approximation to the number of individuals passing up each tree per minute could be calculated. The results are shown in Table VIII.

In both the dieldrin and γ BHC treatments large piles of dead ants were found after one day beneath the treated trees and also along the runways leading to the nests. Very few living ants were seen in the nest areas five days after treatment, although some were alive in the nests. After about 45 days, ants were again active on the soil surface in parts of the colonies radiating from two of the dieldrin-treated trees but none were seen climbing them. At this time some began passing up and down the trees treated with γ BHC and these appeared unaffected. After about 75 days ants began to ascend one of the dieldrin-treated trees.

The DDT treatment was the least effective. At first, and particularly before the insecticide film had dried, the ants appeared to be repelled, for they clustered on each side of the treated band. Piles of dead were found but after 15 days many ants were again foraging in the trees and appeared unaffected.

These preliminary trials show that dieldrin may be useful for controlling *A. custodiens*.

TABLE VIII.

Numbers of worker *A. custodiens* ascending the trunks of mango trees before and after treatment with different insecticide sprays.

Treatment	No. of <i>A. custodiens</i> ascending per tree per minute						
	Before treatment	After treatment					
		2 hrs.	1 day	5 days	15 days	45 days	75 days
Untreated control	782	690	969	506	886	953	665
2% Dieldrin ..	979	11	5	0	0	0	33
2% DDT ..	1100	180	231	32	580	798	603
2% γ BHC ..	1280	10	4	0	2	83	432

Control of *P. punctulata*.

In British East Africa this widely distributed species is by far the most important competitor of *O. longinoda*.

In Zanzibar Island, as previously stated, it may be abundant even in areas of thick ground vegetation. For example, in two small blocks of palms (Selem and Kidichi, fig. 7) *P. punctulata* was nesting at the base of 90% and 94% of the trees respectively. Here, however, with thick ground vegetation, the crowns of 77% and 73% of the palms respectively were occupied by colonies of *O. longinoda*. Although *O. longinoda* was not always abundant in areas of thick vegetation, it was always relatively uncommon where the vegetation was sparse. This is in general agreement with the observations of O'Connor (1950) on the distribution of *O. smaragdina* in the Solomon Islands, and suggests that, although a rich ground vegetation is not deleterious to *P. punctulata*, this ant does not seriously compete with *O. longinoda* when it is present. For this reason a rich ground vegetation should be encouraged. Further reference to vegetation in relation to *P. punctulata* is made below.

Insecticidal control of *P. punctulata* was not attempted. In the Solomon Islands chlordane was destructive to *P. megacephala* (O'Connor, 1950), and in Kenya, dieldrin has proved valuable for *P. punctulata* control in coffee plantations (A. R. Melville, priv. comm.).

Discussion.

There is a striking similarity between the *Theraptus* problem in British East Africa and that of the nearly related Coreid, *Amblypelta cocophaga* China in the Solomon Islands (Lever, 1937; Phillips, 1940; O'Connor, 1950). There, the ant, *Oecophylla smaragdina subnitida* Emery, is a beneficial predator on *A. cocophaga*, but is destroyed by two other ant species, *Pheidole megacephala* and *Iridomyrmex myrmecodiae* Emery which themselves do not prey on the Coreid (Phillips, 1940; O'Connor, 1950). In the Solomon Islands, *Anoplolepis longipes* is said to control *A. cocophaga* but it does not control *Theraptus* in East Africa nor do *A. custodiens* and *P. punctulata*.

It seems that the best method of controlling *Theraptus* or *A. cocophaga* is to encourage *Oecophylla*, and it has been shown (Way, in press) that where this ant occupies more than about 70% of palms, damage by *Theraptus* is relatively slight and probably ceases to be of economic importance. To establish *O. longinoda* in East Africa, it is first necessary to control or suppress the non-beneficial ant species, of which two, *A. custodiens* and *A. longipes*, exterminate *O. longinoda* in the areas which they occupy. The third and most important species, *P. punctulata*, may prevent *O. longinoda* from colonising more than a small proportion of palms.

O. longinoda is an arboreal species and can thrive providing suitable tree or bush vegetation is available. By contrast, soil type and the nature of the ground vegetation may affect the non-beneficial ants, all of which are primarily ground nesting species. The two species of *Anoplolepis* are largely confined to sandy soils bearing a relatively sparse ground vegetation and this suggests that they may be controlled by cultural methods which do not harm the arboreal *O. longinoda*.

P. punctulata is common in all areas occupied by *O. longinoda*, irrespective of the nature of the soil type or the vegetation but *O. longinoda* is often abundant where there is a thick ground vegetation. A similar observation was made in the Solomon Islands by O'Connor (1950) who suggested that *O. smaragdina* was more abundant under these conditions because to reach the ground and colonise neighbouring trees it could use the vegetation growing up the palm trunk thus avoiding the colonies of *P. megacephala* at the base of the trunk. Although this may be a contributory factor, it seems doubtful whether it is the major one. For example, in Zanzibar Island, where palms are closely planted, their fronds often touch, thus allowing *O. longinoda* to reach neighbouring trees without using the ground, but the ant is often scarce or absent. Also, in the Solomon Islands (Phillips, 1940), attempts to encourage *O. smaragdina* by connecting palms with rope and creeper runways have proved unsuccessful. It is true that *O. longinoda* can thrive when prevented from reaching the ground; this was shown by experiments in which the trunks of Clove trees colonised by the ants were grease banded (Way, in press) and also by the fact that they often occur on mangroves in tidal areas. The abundance of both *O. longinoda* and *P. punctulata* in plantations bearing a thick cover of grasses and creepers suggests that, under these conditions, there are different sources of food for each species and that consequently inter-specific competition is relatively slight. It is significant that rich ground vegetation may produce abundant seeds upon which *P. punctulata* feeds and that it also supports Homoptera from which the ants collect honey-dew. Thus it is probably unnecessary for it to seek food in the crowns of palms and other trees. Where ground vegetation is sparse, however, these sources of food are scarce and *P. punctulata* is forced to obtain its food from trees in which it not only destroys established colonies of *O. longinoda* but also the young queens engaged in forming new colonies.

It is of interest that although the influence of a rich ground vegetation on non-beneficial ants differs according to the species, it is always beneficial to *O. longinoda*. Although the establishment and maintenance of the necessary ground cover may not be possible or practicable in many areas, it should be emphasised that either "clean cultivation", or misuse of the soil, in coconut plantations, will encourage the non-beneficial ants, and make control of *Theraptus* by *O. longinoda* even less effective.

Summary.

In the coastal region of British East Africa three ant species, *Anoplolepis custodiens*, *A. longipes* and *Pheidole punctulata* may destroy the ant *Oecophylla*

longinoda which is a valuable predator on the coconut pest *Theraptus* sp. (Coreiidae). The three first-named species do not prey on *Theraptus*, which may severely damage palms occupied by them.

Nesting habits of the three ant species and their behaviour towards *O. longinoda* and certain other insects are described.

O. longinoda has been exterminated in the limited areas occupied by the two *Anoplolepis* species. *P. punctulata* is widespread and is usually common in areas occupied by *O. longinoda* and is also present, though relatively less common, in *A. longipes* areas.

The distribution of the *Anoplolepis* species, particularly *A. custodiens*, is correlated with sandy soils bearing a sparse ground vegetation. Where there are heavy soils or a thick ground vegetation of grasses and creepers the *Anoplolepis* species are absent and *O. longinoda* is usually present. It is suggested that the *Anoplolepis* species are limited by the relatively low temperature of soils shaded from sunlight by thick vegetation.

P. punctulata is not limited by thick ground vegetation, but, under these conditions, *O. longinoda* is also abundant; probably adequate food is available in ground vegetation for *P. punctulata* which thus does not compete for it with *O. longinoda* in the crown of coconut palms and other trees.

Cultural and chemical methods of controlling the harmful ant species are mentioned.

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References.

- CALTON, W. E. (1949). A reconnaissance of the soils of Zanzibar Protectorate.—Tech. Commun. Bur. Soil Sci., no. 46.
- CARTER, W. (1933). The Pineapple Mealybug *Pseudococcus brevipes*, and wilt of pineapples.—Phytopathology, **23**, pp. 207–242.
- KIRKPATRICK, T. W. (1927). The Common Coffee Mealybug (*Pseudococcus lilacinus*, Ckll.) in Kenya Colony.—Bull. Dep. Agric. Kenya, no. 18, 110 pp.
- LEVER, R. J. A. W. (1937). Economic insects and biological control in the British Solomon Islands.—Bull. ent. Res., **28**, pp. 325–331.
- NUTMAN, F. J. & SHEFFIELD, F. M. L. (1949). Studies of the clove tree. I. Sudden-death disease and its epidemiology.—Ann. appl. Biol., **36**, pp. 419–439.
- O'CONNOR B. A. (1950). Premature nutfall of coconuts in the British Solomon Islands Protectorate.—Agric. J. Fiji, **21**, pp. 21–42.
- PHILLIPS, J. S. (1940). Immature nutfall of coconuts in the Solomon Islands.—Bull. ent. Res., **31**, pp. 295–316.
- STRICKLAND, A. H. (1951). The entomology of swollen shoot of cacao. II. The bionomics and ecology of the species involved.—Bull. ent. Res., **42**, pp. 65–103.

- TIDBURY, G. E. & CALTON, W. E. (1950). The use of microplots in a reconnaissance survey of the nutrient status of the soils of Zanzibar Island.—E. Afr. agric. J., **15**, pp. 108–115.
- WAY, M. J. (1951). An insect pest of coconuts and its relationship to certain ant species.—Nature, Lond., **168**, p. 302.
- WAY, M. J. (1953). Studies on *Theraptus* sp. (Coreidae); the cause of the gumming disease of coconuts in East Africa.—Bull. ent. Res., **44**, pp. 657–667.
- WHEELER, W. M. & OTHERS (1922). Ants of the American Museum Congo Expedition. A contribution to the myrmecology of Africa.—Bull. Amer. Mus. nat. Hist., **45**, 1139 pp.
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FIG. 1. Longitudinal section through main chamber of an *A. longipes* nest at Mazizini, Zanzibar Island.

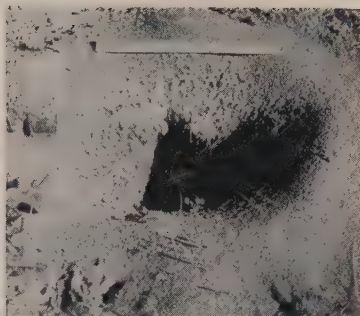


FIG. 2. Main entrance to nest of *A. longipes* at Marhubi, Zanzibar Island.



FIG. 3. Longitudinal section through nests of *A. custodiens* at Marhubi, Zanzibar Island.

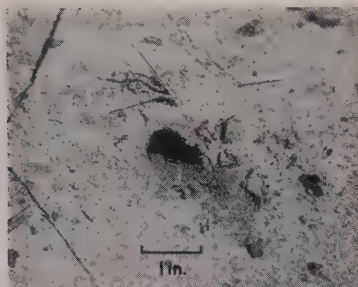


FIG. 4. Entrance to nest of *A. custodiens* near Mbagalla, Tanganyika.



FIG. 5. Adults and nymphs of *Aspidoproctus armatus* on the trunk of *Cassia* sp. near Mbagalla, Tanganyika. Workers of *A. custodiens* were collecting the honey-dew excreted by these insects.

A COMPARATIVE STUDY OF ANTI-LOCUST BAITS, WITH SPECIAL REFERENCE TO BASE MATERIALS.

By H. B. N. HYNES.

Department of Zoology, University of Liverpool.

EMM

During the large-scale campaigns against the Desert Locust, *Schistocerca gregaria* (Forsk.), between 1942 and 1947 (Uvarov, 1951) I made a number of field tests, in Kenya and Italian Somalia, for the East African Anti-Locust Directorate. These tests were designed primarily to assess the suitability of various base materials for use in the campaigns, but taken together they also indicated some factors which may control the "acceptability" (Gunn, 1952) of materials. A further series of tests was designed to investigate this, but the urgent duties of anti-hopper campaigns prevented my making them before I left East Africa towards the end of 1946.

Recently Dr. R. C. Rainey of the Desert Locust Survey, after seeing my reports on the files of the East African Anti-Locust Directorate in Nairobi, has suggested that publication of my method, and of such results as I did obtain, would be of value to anti-locust workers. This paper is the result of that suggestion, and I wish gratefully to acknowledge the assistance given to me by Dr. Rainey and Dr. B. P. Uvarov, F.R.S., in its preparation.

It should perhaps be emphasised here that the present study was confined to wet baits. Recent work by Joyce (1952) and Gunn (1952) has shown that some of the better materials can be used as dry baits for the Desert Locust, and Dr. Uvarov has informed me that dry baits are now in general use for this species.

Methods of Testing Baits.

The various methods for testing baits fall into two categories, which both include field and laboratory techniques.

Acceptability tests.

Observations of the insects as they feed on the bait, or study of the amount eaten have been described by Shotwell (1942) and Joyce (1952) who counted the number of insects feeding on pans of bait laid out in the field. Du Plessis and Botha (1939) used a similar technique on migratory bands of the Brown Locust, *Locustana pardalina* (Wlk.), which had been confined inside metal barriers. Notley (1946) made similar counts of hungry hoppers of the Desert Locust feeding on baits in cages, and Gunn (1952) made a subjective estimate of the relative acceptability of baits based on the reactions of locust hoppers in the field. Du Plessis and Nolte (1941) measured the areas of maize leaf, previously dusted with various substances, which were eaten by caged hoppers in unit time.

Percentage kill tests.

Most of these are based on what Shotwell calls the "plot-and-cage" method. Areas containing hoppers are baited, and after the insects have fed, samples are collected and caged, and the percentages that die are recorded. This technique is difficult with mobile bands of locust hoppers, but Faure and Jacot-Guillarmod (1940) overcame this by confining the hopper-bands inside metal barriers. Joyce (1952) and Gunn (1952) have attempted to estimate percentage kill under field

conditions by baiting bands of locust hoppers and then studying the baited area and surrounding land after an interval.

The obvious objection to tests made in cages is that the insects do not have their normal choice of food, and are brought into very close contact with the bait. This is particularly true if the insects are first made hungry, as was done by Notley (1946). The plot-and-cage method is also open to the criticism that it is very difficult to obtain a random sample of poisoned hoppers, as there is a tendency for the more heavily dosed specimens to become sluggish very soon after feeding. It is also possible to criticise the acceptability method on the ground that one cannot distinguish between hoppers which are feeding and those which are merely sitting on the bait, and that it is difficult to count hoppers without disturbing them.

Nevertheless, it would appear that acceptability tests are as likely to give as good an indication of the efficacy of baits as any other method, particularly if they are performed in the field, and if one is sufficiently sure of the toxicity of the poison used.

In the present investigation, the various baits were tested on bands of well-grown hoppers of the Desert Locust under entirely natural conditions. The tests were performed in the Turkana district of Kenya and in Somalia, and all were made before 10 a.m. or after 4 p.m., when the hoppers were actively marching and feeding. Gregarious hoppers of this species march steadily in one direction, but break up into anastomosing streams round patches of vegetation, bushes, etc.

For each test a covered truck was parked at the front of a band, at a point where several broad and even hopper streams could be seen at once. Then a line of small plots of the several baits under test was quickly laid across the middle of each stream, where the hopper density was fairly even. Each line contained one plot of each bait, and the positions of the various baits in each line were chosen at random. Each plot was about 1 sq. ft. in area and 1 foot from the next plot, and the bait was spread in a layer about 1/10 inch thick.

About ten minutes after the laying of the bait, when the hoppers had recovered from the disturbance, those which had stopped on each plot were counted by observing them with binocular field glasses from the shelter of the truck. The counts were repeated at five-minute intervals until three, or in the earlier tests often more, had been made. The test was continued until at least five, or in the later tests ten, differently randomised lines of bait had been studied.

The truck, although not essential, was found to make a convenient observation post, enabling one to remain fairly close to the plots and to view them from above. The lines were not more than 20-30 yards from the truck, and the hoppers were thus fairly easy to count. The truck also had the advantage of hiding the observer from the hoppers, which were very easily disturbed by small movements, and it could be readily driven to another site for laying further lines of plots as the test proceeded.

For assessment of the results, the sum of the counts for each plot was regarded as the number of hoppers feeding on that plot, and these plot totals were subjected to Student's Variance Method for the assessment of randomised block lay-outs (Cowan, 1934; Shotwell, 1942). Differences between the total numbers of hoppers feeding on each bait were regarded as significant at $P < 0.05$ and highly significant at $P < 0.01$.

In one of the earlier experiments nine baits were used in one test, but this was found to be unwieldy because many of the hopper streams were too narrow, and often only one line could be laid at a time. The number of baits used in each test was therefore reduced to five. Joyce (1952) also found the same difficulty when attempting to study as many as 30 small trays of bait laid across a single hopper-stream. Some of his trays were outside the stream altogether,

and those near the edge, where the density of hoppers is always lower, collected only a few hoppers. This resulted in such varied tray totals that only one of his results was statistically significant. He found for example no significance between the acceptability of wheat bran and crushed maize (maize meal), which the present study showed, as stated below, to differ considerably in attractiveness.

In another early test eight counts were made on each plot. This had no effect on the results, as was shown by the fact that the plot totals of the first three counts gave the same result as the plot totals of the last five counts. The number of counts per plot was therefore reduced in later experiments.

Materials Tested.

The following base materials were tested:—

Maize meal consisting of ground white maize.

Maize bran, wheat bran.

Cottonseed husk derived from cotton seed from which the long staple cotton lint had been removed by ginning and in which there remained a fair amount of broken endosperm.

Rice bran which contained a fair amount of broken rice grain, *rice polishings, rice husk.*

Groundnut husk.

Bagasse (sugarcane waste), *sugarcane trash, millet stalk, maize stalk and corn cob* (maize cob). These five materials had been broken up into small pieces in a hammer mill.

Sawdust made from the wood of *Podocarpus*.

Coffee husk (coffee parchment), *buni husk*, which is coffee husk from which the flesh of the coffee berry has not been removed before drying, *old buni husk* which had been composted for several months after being mixed with sodium arsenite as locust bait, and from which presumably the sugar, starch etc. present in the dried fruit pulp had been lost by fermentation.

Shea butter seed cake and *dom palm nut residue*, which were both the remains of nuts from which it was understood the oil had been extracted by pressing. The dom nut material used would appear to have been different from the dom flour later studied by Gunn (1952), which was stated to be the by-product of the manufacture of buttons from the kernels. Not only was my material clearly much less acceptable than his, but it was too coarse and hard to be described as a flour. Unfortunately I have no information about its origin.

Some authors have found that sodium arsenite adds to the acceptability of baits (*e.g.* Faure, 1935), others that it is without effect (du Plessis & Botha, 1939), and Shotwell (1942) considers that arsenic may be repellent. Because of this doubt all the materials tested in the present investigation were mixed with 1½–2 per cent. by weight of sodium arsenite, which was at that time the poison in general use. They were also wetted to the consistency of wet baits as used in the field. That is, they were made wet enough to adhere in balls when squeezed in the hand but not wet enough to drip. No substances were tested dry, although it was many times observed that the most acceptable materials remained acceptable long after the others had been abandoned by the hoppers, and that they were often completely consumed. In four tests molasses was added to some of the materials at the rate of 1 pint (about 0.6 litres) or more per sack, the sacks being standard 100 kilogram grain sacks. The different materials, of course, differed widely in density.

Results.

Table I shows the details of the tests, the total number of hoppers feeding on each bait and the standard error of the differences between these totals. In test 5 certain mixtures of baits were also included but, as these are not relevant to the present discussion, they are not included here, although their plot totals have been used in calculating the standard error of difference between bait totals.

Base Materials.

With the data given in Table I it is possible to compare the acceptability of any base materials used in any one test. Each material is shown below as "better than" or "worse than" the other materials against which it was tested. The number of the experiment in which the result was obtained is also shown. Where *P* was less than 0.01 the experiment number is given in italics. The term "equal to" indicates that no significant difference was found.

Bagasse	worse than	maize bran 1, 7, 9, maize meal 1, 9, rice bran 6, cottonseed husk 6, 10, wheat bran 9, 10, 11, 12, 13, 14, 15, buni husk 14, rice polishings 15.
	equal to	groundnut husk, 1, 13, sugarcane trash 1, 7, rice bran 1, 10, coffee husk 1, 2, 9, 10, old buni husk 6, millet stalk 1, 2, 7, 11, shea seed cake 6, sawdust 12, rice husk 13, maize stalk 15.
	better than	corn cob 1, 12, sugarcane trash 11, coffee husk 7, 11, 12, 13, 14, 15, dom nut residue 14.
Buni husk	worse than	wheat bran 14.
	better than	dom nut residue 14, bagasse 14, coffee husk 14.
Coffee husk	worse than	maize bran 1, 7, 9, sugarcane trash 7, 11, bagasse 3, 11, 12, 13, 14, 15, millet stalk 7, 11, maize meal 1, 9, wheat bran, 9, 10, 11, 12, 13, 14, 15, cottonseed husk 10, buni husk 14, rice polishings 15.
	equal to	groundnut husk 1, 13, sugarcane trash 1, bagasse 1, 2, 9, 10, millet stalk 1, rice bran 1, 10, corn cob, 1, 12, sawdust 12, rice husk 13, dom nut residue 14, maize stalk 15.
Corn cob	worse than	maize bran 1, maize meal 1, groundnut husk 1, bagasse 1, 12, wheat bran 12.
	equal to	sugarcane trash 1, millet stalk 1, rice bran 1, coffee husk 1, 12, sawdust 12.
Cottonseed husk	worse than	wheat bran 10.
	equal to	rice bran 6.
	better than	rice bran 3, 10, bagasse 6, 10, old buni husk 3, 6, shea seed cake 6, coffee husk 10.
Dom nut residue	worse than	wheat bran 14, bagasse 14, buni husk 14.
	equal to	coffee husk 14.
Groundnut husk	worse than	maize bran 1, maize meal 1, wheat bran 13.
	equal to	bagasse 1, 3, sugarcane trash 1, millet stalk 1, rice bran 1, coffee husk 1, 13, rice husk 13.
	better than	corn cob 1.
Maize bran	worse than	wheat bran 9, maize meal 9.
	equal to	maize meal 1.
	better than	groundnut husk 1, bagasse 1, 7, 9, sugarcane trash 1, 7, millet stalk 1, 7, rice bran 1, coffee husk 1, 7, 9, corn cob 1.

Maize meal	equal to	maize bran 1.
	better than	groundnut husk 1, bagasse 1, 9, sugarcane trash 1, millet stalk 1, rice bran 1, corn cob 1, coffee husk 1, 9, wheat bran 9, maize bran 9.
Maize stalk	worse than	wheat bran 15, rice polishings 15.
	equal to	bagasse 15, coffee husk 15.
Millet stalk	worse than	maize bran, 1, 7, wheat bran 11.
	equal to	coffee husk 1, groundnut husk 1, bagasse 1, 2, 7, 11, rice bran 1, corn cob 1, sugarcane trash 1, 7, 11.
	better than	coffee husk 7, 11.
Old buni	worse than	rice bran 6, cottonseed husk 3, 6.
husk	equal to	bagasse 1, rice bran 3, shea seed cake 6.
Rice bran	worse than	maize bran 1, maize meal 1, cottonseed husk 3, 10, wheat bran 10.
	equal to	bagasse 1, 10, coffee husk 1, 10, old buni husk 3, cottonseed husk 6, groundnut husk 1, sugarcane trash 1, millet stalk 1, corn cob 1.
	better than	bagasse 6, old buni husk 6, shea seed cake 6.
Rice husk	worse than	wheat bran 13.
	equal to	groundnut husk 13, bagasse 13, coffee husk 13.
Rice	better than	wheat bran 15, bagasse 15, maize stalk 15, coffee husk 15.
polishings		
Sawdust	worse than	wheat bran 12.
	equal to	bagasse 12, corn cob 12, coffee husk 12.
Shea seed	worse than	rice bran 6, cottonseed husk 6.
cake	equal to	bagasse 6, old buni husk 6.
Sugarcane	worse than	maize bran 1, 7, maize meal 1, wheat bran 11, bagasse 11.
trash	equal to	coffee husk 1, groundnut husk 1, bagasse 1, 7, millet stalk 1, 7, 11, rice bran 1, corn cob 1.
	better than	coffee husk 7, 11.
Wheat bran	worse than	rice polishings 15, maize meal 9.
	better than	maize stalk 15, buni husk 14, dom nut residue 14, maize bran 9, bagasse 9, 10, 11, 12, 13, 14, 15, coffee husk 9, 10, 11, 12, 13, 14, 15, cottonseed husk 10, rice bran 10, sugarcane trash 11, millet stalk 11, sawdust 12, corn cob 12, groundnut husk 13, rice husk 13.

It will be seen that some substances are shown as having been both equal to and significantly different from another substance, but in no instance was a base material found to be both better than and worse than another. It can be assumed therefore that these anomalies have occurred merely because some experiments were less conclusive than others.

From the above tabulation of results it is possible to divide the various base materials into five classes of decreasing acceptability as follows:

- (1) *better than wheat bran*—maize meal and rice polishings.
- (2) *wheat bran*
- (3) *worse than wheat bran and better than bagasse*—maize bran, cottonseed husk, buni husk and rice bran.

TABLE I.

Details of the individual field tests.

Expt. no.	Locality and date	Type of country	Hopper stage	No. of replications	No. of counts / plot	Bait base material	Pints molasses / bag	Total no. of hoppers feeding	Standard error of difference
1	Wardere, Somalia 28 & 29. vi. 45	fairly dense high bush with bare red sand	c. 90% 3rd c. 10% 4th	9	3	maize bran maize meal groundnut husk bagasse sugarcane trash millet stalk rice bran coffee husk corn cob	- - - - - - - -	1805 1785 820 815 720 570 410 300 245	265
2	Wardere, Somalia 29. vi. 45	open bush with some herbs	4th	7	3	millet stalk bagasse coffee husk	- - -	265 225 120	59
3	Loiyapuya, Turkana, Kenya 29. viii. 44	open lava gravel some bushes	5th	8	5	cottonseed husk rice bran rice bran old buni husk	- - 1 -	923 524 452 283	130
4	Wardere, Somalia 26. vi. 45	dense high bush with bare red sand	c. 90% 3rd c. 10% 4th	10	2	bagasse " " "	8 4 2 1 -	786 326 285 281 133	67
5	Wardere, Somalia 30. vi. 45	low bush, bare grey dust	5th	8	3	" "	- 1	241 137	140
6	Loiyapuya, Turkana, Kenya 28. viii. 44	open lava gravel with scattered grass and bushes	5th	5	8	rice bran cottonseed husk bagasse old buni husk shea seed cake	- - - - -	805 780 196 93 88	97
7	Wardere, Somalia 30. vi. 45	open bush with some herbs and bare red sand	4th	5	3	maize bran bagasse sugarcane trash	- - -	670 210 190	49

8	Loiyapunya, Turkana, Kenya 27. viii. 44	open area grazed with scattered grass and bushes	5th	5	5	" " " "	4 2 1 —	718 584 511 338	132
9	Balad, Somalia 17. xii. 45	scattered trees and long grass; black soil	5th	10	3	maize meal wheat bran maize bran bagasse coffee husk	— — — — —	711 540 386 136 70	66
10	Balad, Somalia 18. xii. 45	scattered trees and long grass; black soil	5th	10	3	wheat bran cottonseed husk bagasse rice bran coffee husk	— — — — —	735 258 108 79 41	43
11	Balad, Somalia 18. xii. 45	dense bush with rough grass and bare black soil	5th	10	3	wheat bran bagasse millet stalk sugarcane trash coffee husk	— — — — —	921 168 163 122 54	23
12	Balad, Somalia 19. xii. 45	dense bush with bare black soil	5th	10	3	wheat bran bagasse sawdust corn cob coffee husk	— — — — —	921 166 108 49 37	38
13	Afgoi, Somalia 20. xii. 45	medium bush with some grass and bare black soil	5th	10	3	wheat bran bagasse rice husk groundnut husk coffee husk	— — — — —	584 95 56 48 39	24
14	Afgoi, Somalia 21. xii. 45	medium bush with some grass and bare black soil	5th	10	3	wheat bran buni husk bagasse dom nut residue coffee husk	— — — — —	691 216 145 65 30	30
15	Afgoi, Somalia 21. xii. 45	medium bush with some grass and bare black soil	5th	10	3	rice polishings wheat bran bagasse maize stalk coffee husk	— — — — —	878 544 170 160 43	61

- (4) *equal to bagasse and better than coffee husk*—sugarcane trash, millet stalk and probably also maize stalk which closely resembles these materials, and which in test 15, the only one in which it was included, only just failed to be significantly better than coffee husk.
- (5) *not significantly different from coffee husk*—groundnut husk, corn cob, sawdust, rice husk, dom nut residue, and almost certainly old buni husk, and shea seed cake, neither of which was tested directly against coffee husk.

These results are in general agreement with those of Shotwell (1942), Lea (1935), Mossop (1933), Faure and Jacot-Guillarmod (1940) and Notley (1946), who tried several of these materials on various species of locusts and grasshoppers. The only discrepancies are that Notley found that corn cob was more acceptable than bagasse and coffee husks and that sawdust was less acceptable than coffee husks. It is probable that the sawdust he tested was not made from the same kind of timber, and Major Notley kindly informed me by letter in October 1945 that it was possible that his sample of corn cob was in some way contaminated. Only a small sample was milled for his tests, and this may have been done in an uncleaned mill.

Consideration of this classification of certain base materials shows that there is some relationship between the acceptability and the chemical and physical constitution of the materials. Class 5 is composed of hard, lignified materials, Class 4 of materials that are relatively soft, and nearly pure cellulose, and class 1 is composed of starchy materials. Classes 2 and 3 contain materials in which a certain amount of starch is present. This perhaps indicates that heavy lignification renders unacceptable certain possible base materials, and that the presence of starch increases acceptability, but there may well be other factors involved. It would seem that further investigations along these lines might give valuable results.

Addition of molasses.

The necessity for sweetening agents or other taste improvers has been extensively investigated in U.S.A. and South Africa. In America, Shotwell (1942) has shown that molasses and a variety of other substances, including some salts, do not add to the acceptability of bran. In South Africa, du Plessis and Botha (1939), Coaton (1939), Faure and Jacot-Guillarmod (1940) and du Plessis and Nolte (1941) showed that the addition of molasses and sugar to bran, and of sugar to sawdust, did not add to their acceptability. Moreover, Mally (1923) has suggested that treacle causes precipitation of arsenic and so results in bad mixing of arsenical baits. Its use has, however, persisted in many countries both with bran baits and other substances.

The relative acceptability of the baits investigated in the present study, with and without molasses, is compared below, in the same way that the various base materials were compared. The letter M indicates that molasses was added at the rate of 1 pint/bag, 2M at the rate of 2 pints/bag etc.

cottonseed	worse than	cottonseed husk	3M	8
husk		" "	4M	8
	equal to	" "	M	8
		" "	2M	8
rice bran	equal to	rice bran	M	3
bagasse	worse than	bagasse	M	4
			2M	4
			4M	4
			8M	4
	equal to	bagasse	M	5

Although molasses was added in only a few tests it is clear that addition of 1 pint per bag increased the acceptability of bagasse, but that this amount had no significant effect on the more acceptable base materials rice bran and cottonseed husks. It was found that the acceptability of cottonseed husk was not increased until 3 or 4 pints of molasses/bag had been added. It would seem therefore that addition of molasses does increase the acceptability of baits, but that its effect is much greater when used with less acceptable materials.

This conclusion seems to be in agreement with the findings of other workers that molasses does not add to the acceptability of bran baits, and to be borne out by the fact that in U.S.A. the addition of molasses to sawdust baits was recommended (Parker, 1939). It also agrees with Notley's (1946) findings that molasses increased the acceptability of corn cob, bagasse, coffee husks and sawdust, but did not alter that of wheat bran.

Water absorption and rate of drying.

Husain and Mathur (1936) have shown that adults of *S. gregaria* will eat almost anything if it is wet, and Notley (1946) has stressed the importance of water-absorbing ability of base materials for baits. Also several locust-control officers working, during the 1942-47 campaigns against the Desert Locust, with baits made of cottonseed husk, coffee husk, bagasse and millet stalk, suggested to me that molasses was valuable because it kept the bait acceptable for a longer period.

These remarks of course apply only to base materials which are not sufficiently acceptable for use as dry baits.

It is of interest to note that simple tests performed at Mogadishu showed that the amount of water absorbed per unit volume, and hence per unit area of spread bait, was not at all correlated with the acceptability of the fresh bait. The rate of drying in the sun, as determined by repeated weighings at fixed intervals, was found to be fairly uniform for all baits, although slower in finely divided and very starchy materials and in all materials when they were nearly dry. The time taken to reach complete dryness was therefore fairly directly correlated with the amount of water initially absorbed, so that a bait which absorbed more water per unit volume, would presumably be fed upon by more hoppers than an equally acceptable but less water-absorbent bait.

These tests also showed that addition of salt and molasses to baits delayed their rate of drying, but that soda did not. The effect of molasses was, however, very slight, unless very large amounts, equal to about five times the normal rate of 1 pint per bag, were used.

Summary.

A simple, but reliable, technique is described for the study of the acceptability to locust hoppers of poison baits under field conditions.

This was applied to the study of a number of vegetable materials which might be used for the manufacture of wet baits to destroy hoppers of the Desert Locust.

The materials tested could be divided into five classes of acceptability, and consideration of these indicated that heavy lignification detracted from acceptability while the presence of starch added to it.

It was shown that molasses adds to the acceptability of only the least acceptable materials unless very large amounts are used.

Simple tests showed that acceptability was not correlated with water-absorbing properties, that the rate of drying of bait was dependent on the amount of water initially absorbed, and that molasses influences the rate of drying only when large amounts are added.

References.

- COATON, W. G. H. (1939). Field tests of poison bait against hoppers of the red locust, 1935-6.—J. ent. Soc. S. Afr., **2**, pp. 115-133.
- COWAN, F. T. (1934). Application of the variance method to the comparison of grasshopper baits.—J. econ. Ent., **27**, pp. 705-713.
- DU PLESSIS, C. & BOTHA, D. H. (1939). Preliminary field experiments on the attractiveness of certain chemicals and bait carriers to the hoppers of the brown locust.—J. ent. Soc. S. Afr., **2**, pp. 74-92.
- DU PLESSIS, C. & NOLTE, M. C. A. (1941). Laboratory experiments on the improvement of poison baits for hoppers of the red locust: 1936-37.—Sci. Bull. Dep. Agric. S. Afr., no. 227, 44 pp.
- FAURE, J. C. (1935). Is arsenite of soda an attractant for locusts?—Rep. Interstate Locust Conf. Pretoria 1934, pp. 64-66.
- FAURE, J. C. & JACOT-GUILLARMOD, C. F. (1940). Field experiments on poison bait against hoppers of the red locust: 1936-37.—Sci. Bull. Dep. Agric. S. Afr., no. 211, 52 pp.
- GUNN, D. L. (1952). Field tests of dry baiting against the desert locust, *Schistocerca gregaria* (Forsk.).—Bull. ent. Res., **42**, pp. 675-690.
- HUSAIN, M. A. & MATHUR, C. B. (1936). Studies on *Schistocerca gregaria* (Forsk.). III. Why locusts eat wool.—Indian J. agric. Sci., **6**, pp. 263-267.
- JOYCE, R. J. V. (1952). Field trials with various dry baits against the desert locust, *Schistocerca gregaria* (Forsk.).—Bull. ent. Res., **42**, pp. 691-696.
- LEA, A. (1935). Investigations on the red locust in Natal in 1934.—Rep. Interstate Locust Conf. Pretoria 1934, pp. 50-62.
- MALLY, C. W. (1923). Arsenite of soda as a locust poison.—Agric. J. Un. S. Afr., **6**, pp. 220-232.
- MOSSOP, M. C. (1933). Biological notes on the red locust, *Nomadacris septemfasciata*, Serv.—Bull. Dep. Agric. S. Rhod., no. 904, pp. 25-47.
- NOTLEY, F. B. (1946). Tests on locust baits in Somalia.—Bull. ent. Res., **37**, pp. 89-94.
- PARKER, J. R. (1939). Grasshoppers and their control.—Fmrs.' Bull. U.S. Dep. Agric., no. 1828, 37 pp.
- SHOTWELL, R. L. (1942). Evaluation of baits and bait ingredients used in grasshopper control.—Tech. Bull. U.S. Dep. Agric., no. 793, 51 pp.
- UVAROV, B. P. (1951). Locust Research and Control 1929-1950.—Colon. Res. Publ., no. 10, 67 pp.
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AN EXPERIMENT ON THE CONTROL OF TSETSE
(*GLOSSINA PALPALIS* R.-D.)
IN HIGH FOREST OF WEST AFRICA.

By B. J. A. NOWOSIELSKI-SLEPOWRON, B.Sc.

Scientific Assistant, Department of Tsetse Control, Gold Coast.

Kumasi (fig. 1), the capital of Ashanti in the Gold Coast, is growing rapidly. In the 1931 census its population was 36,284, in the 1948 census it was 78,483, and it is still expanding rapidly both in population and size. This creates housing problems and various schemes have been undertaken to ease the situation. New houses and shopping centres, with the roads that serve them, are continually extending it, and schools, recreational centres, saw mills etc. are being constructed, usually at a small distance from the existing boundaries of the town. This rapid building programme creates opportunities for large forces of migrant labourers, mostly from the Northern Territories and surrounding French territory and these, sometimes with their families, come to work and swell the already congested population of Kumasi.

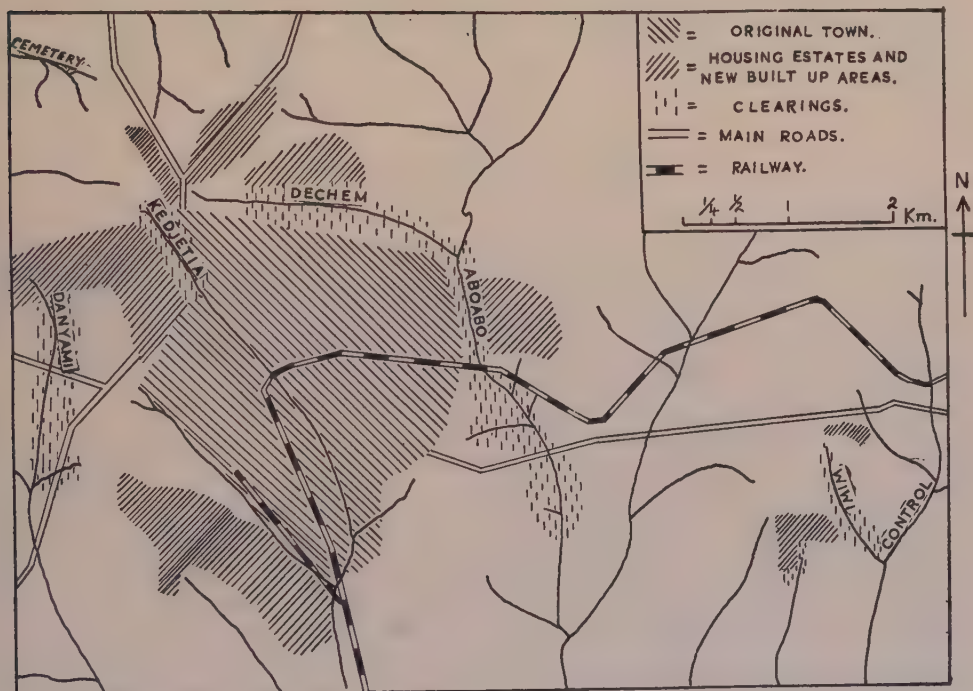


Fig. 1.

The labourers, also many of the travelling traders, may come from, or cross parts of country where sleeping sickness exists. Consequently they may be infected with *Trypanosoma gambiense* sleeping sickness, the slow development

of which has been described by Cooke, Gregg, and Manson-Bahr (1937), and thus unknowingly bring the disease to Kumasi.

The new houses which are being constructed are usually built on the ridges, the valleys between remaining as thickly covered as ever either with primary or secondary forest, which is the habitat of *Glossina palpalis* (R.-D.), *G. pallicera* Bigot, *G. nigrofusca* Newst. and possibly other species of *Glossina* which are known or potential vectors of trypanosomiasis. The built-up ridges meet, and the valleys in between are often traversed by a road or a path, thus artificially creating a situation of considerable potential danger. The tsetse vectors being confined to unnaturally isolated and restricted habitats, a high degree of man-fly contact is maintained along the roads and paths and around the periphery of the uncut bush. Cases of trypanosomiasis are being diagnosed at Abrepo, the treatment centre north of Kumasi.

In view of this situation, Dr. K. R. S. Morris, the then Director of the Department of Tsetse Control, devised and initiated an experimental clearing in Kumasi with the object of eliminating these dangerous isolated foci of *Glossina* with the minimum clearance of bush, and of estimating the degree of control of the tsetse within it.

The Climate, Country and Vegetation.

The town itself lies in hilly country and is surrounded by semi-deciduous rain forest, with very dense under-growth or with cocoa trees. Between the housing estates in the vicinity of the town, farms are to be found which range from those under intensive cultivation to those which have been abandoned and have become overgrown by secondary forest. Both types of vegetation provide habitat for at least one species of *Glossina*, *G. palpalis*, and possibly others.

TABLE I.

Meteorological data; monthly averages.
Readings at 09.00 hrs. G.M.T.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Rainfall in inches	1.00	1.75	4.50	5.75	6.75	9.50	4.50	2.50	5.75	7.75	4.75	1.75
R. H. %	82	80	78	79	79	82	83	83	83	82	79	82
Temp. in °F.	78	82	83	85	84	82	78	76	79	80	82	80

The climate throughout the year is constant in temperature and humidity with the rainfall showing clear periodicity. The average monthly records show that the temperature varies from 76°F. in August to 85°F. in April, the relative humidity from 78 per cent. in March to 83 per cent. in July, August and September, and the rainfall from 1.00 inch in January to 9.50 inches in June. It can also be seen that the relation between the rainfall and the humidity taken at 09.00 hrs. seems to be obscure (see Table I). Unfortunately data for the percentage relative humidity at 15.00 hrs. could not be obtained.

Dechem Valley Clearing.

The clearing of the Dechem valley (fig. 1) was started in July 1950 and until January 1951 it was carried out with only infrequent scientific supervision. Nevertheless, the African officer in charge of the work had had long experience of clearing in the Northern Territories (Morris, 1946; Morris & Morris, 1949), and so was able to carry out the initial stages of the clearing completely satisfactorily.

Dechem valley consisted of overgrown farm-land with bush up to 20 ft. high. The technique of clearing was to cut with cutlasses and axes strips of vegetation on both sides of the stream to a total depth of 250–300 yards, dig out the roots, pile up cut bush and roots for drying, and finally to burn the piles. Large trees, especially those with clean boles, were not cut down. To reduce the costs it was decided to leave as many of the larger trees as possible without losing the effectiveness of clearing. These operations, therefore, fall into the category of discriminative clearing, in which well trained supervisors were employed to judge the amount of bush to be cut or left. It was found that recorders, fly-boys and headmen with a good knowledge of tsetse habitat acquired in the savannah country, could soon be trained to apply their knowledge to the local conditions around Kumasi and to carry out this discriminative clearing efficiently.

Tsetse traps were used to evaluate the effect of clearing in fly reduction and proved to be most valuable indicators of the persistence of one or two foci of tsetse, which had been missed during the first clearing operations. Since discriminative clearing is based on personal judgement it is valuable to have an entirely automatic, impersonal check, such as can be provided by traps, to ensure that unsuspected fly foci are detected and subsequently cleared up.

The success or failure of the clearing was judged by placing 20 traps of the type described by Morris and Morris (1949) at intervals along the Dechem stream at the beginning of clearing operations, and catches in these traps were recorded three times a week. To supplement the trap-round, a fly-round was set out in December 1950. The traps were sited on the principles laid down by Morris and Morris (1949) and before records were taken some of them had to be re-sited. To facilitate the presentation of the data, traps were grouped, and records of the catches of each trap group in relation to the progress of clearing, are presented in Table II.

TABLE II.
Catches recorded in traps.

Months 1950	Gp. I 3 traps	Gp. II 4 traps	Gp. III 3 traps	Gp. IV 2 traps	Gp. V 3 traps	Gp. VI 3 traps	Gp. VII 2 traps	Rainfall in inches
August	17	3	18	15	47	40	1	4.68
September	6	6	26	4	19	97	28	3.38
October	0	7	6	9	25	89	18	7.64
November	1	8	7	3	14	63	4	3.01
December	0	3	4	2	0	38	8	0.88
1951								
January	0	1	3	3	6	9	4	1.24
February	0	1	3	2	1	11	2	4.21
March	1	4	8	1	6	11	9	5.49

The heavy line indicates the progress of clearing in relation to each group of traps.

It may be seen from Table II and fig. 2, that a reduction in number of flies recorded from a trap group is apparent after clearing the bush in its vicinity. There is often no sharp and clear-cut reduction, but a gradual fall to a much

lower level. This is due to the fact that flies come from the head of the clearing to the traps at the nearby clearing, flies attack the numerous labourers engaged in the work of clearing and thus do not enter the traps, and the influence of rainfall and humidity which affects the numbers and possibly the activity of the fly.

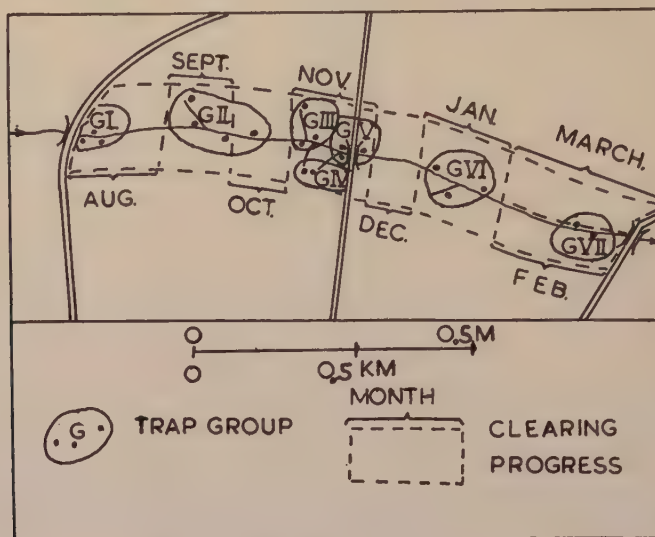


Fig. 2.—Sketch plan of Dechem valley.

Dechem valley is traversed by three roads, the two outer ones forming the western and eastern boundaries of the clearing whilst the middle one divides it into nearly equal parts. The stream flows eastwards. The fly-round worked for one day a week in the west part, called Dechem I (corresponding to trap groups I–V), one day a week in the east part called Dechem II (corresponding to trap groups VI–VII), and one day a week at a comparable uncleared stream, called the Cemetery Stream. The records of the fly-round are presented in Table III.

TABLE III.

Fly-round record. Dechem valley and control (Cemetery Stream).

Month 1951	Dechem I	Dechem II	Control	Rainfall in inches
January	1	9	24	1.24
February	2	6	40	4.21
March	7	21	60	5.49

All catches averaged to 10 boy-days.

The increase in number of flies recorded in the control shows the influence of the rainfall; the same can be seen in the Dechem rounds.

At the end of March 1951 it was decided that the results justified extending the clearing into other valleys, with similar vegetation associations, surrounding

the growing town of Kumasi, and a five-year plan was put forward. The five-year plan, when completed, will eliminate all isolated fly habitats in and around Kumasi, between the town and housing estates already built, and those to be built in the near future. It is hoped to surround Kumasi with three more or less complete fly-free belts of valleys, the ridges separating them being utilised mostly for buildings.

Up to the time of writing about a sixth of the proposed clearing has been completed. It may be said that in most cases the reduction of tsetse achieved by this method of control has been about 80 per cent. or more, often around 90 per cent., but in the case of Kedjetia the eradication has been for all practical purposes complete.

Kedjetia.

In January 1951, after the first results of the Dechem clearing had been assessed, and found to be successful, clearing of Kedjetia valley was started. This was potentially one of the most dangerous isolated habitats of *Glossina* in Kumasi. Its potential danger will be best appreciated from the following description.

Kedjetia was overgrown with dense if patchy bush, 6–20 ft. high, with many tsetse breeding in it, and feeding all along its periphery. Its eastern boundary is a road, the main direct route to the Northern Territories, with constant motor, cycle, pedestrian and cattle traffic. To the north-east there is a cinema, a slaughter house and a school with two more schools on its northern boundary. To the south-west there is a hospital and a leprosy treatment centre; and to the south, the centre of the town with a very busy lorry park in its immediate vicinity, and the usual complement of vendors, traders and stalls.

A high population of *Glossina* was not only maintained by the favourable habitat and conveniently close feeding grounds, but it was also periodically reinforced by flies brought in by the cattle driven in for slaughter.

This clearing was completed by the end of March 1951. Several fly-rounds made in the cleared valley failed to catch a single fly, although a few flies were taken from cattle which had just arrived at the slaughter house. The inference from this was that any introduced flies had failed to maintain themselves in the completely changed vegetational complex.

Aboabo.

The Dechem stream is a tributary of a larger stream called Aboabo which flows south along the eastern boundary of Kumasi and separates it from the Aboabo housing estate. The Aboabo valley is also an isolated habitat, and as such had to be dealt with. Some of the houses on both sides of the stream were as close as 20 yards to the nearest permanent habitat of *Glossina*.

The vegetation in this valley was similar to that of Dechem, and the same discriminative clearing procedure was adopted. Again a reduction of tsetse was recorded after the clearing was completed in March 1952.

Danyami.

The clearing of the valley of this stream, on the western boundary of Kumasi, which divides Kumasi from the Suntreso Housing Estate, was commenced in April 1952. The clearing of this valley will complete the first, inner, belt of fly-free valley around Kumasi.

Regional College of Technology—Wiwi Stream.

In May 1951 the Department of Tsetse Control received a request from the authorities of the Regional College of Technology to assist and advise on tsetse

control in the immediate vicinity of the College, which at that time was under construction. The labourers and their supervisors were complaining of tsetse-flies.

The College, about four miles from Kumasi, consists of a self-contained unit of lecture halls, bungalows, dormitories and a small housing estate; it lies on high ground surrounded by streams with very dense secondary bush, and high forest not more than half a mile distant on the south, east and north sides. This was not a very hopeful proposition for tsetse eradication, in view of the close proximity of such an extensive habitat of *G. palpalis*. As the building programme progressed, parts of the bush nearest to the houses and those between the existing and planned housing blocks would become isolated habitats and this might result in these areas becoming foci of infection.

After consultation with Dr. K. R. S. Morris, the then Director of the Department of Tsetse Control, it was decided to attempt to prevent such a situation arising and, as the clearing technique had been successful in Kumasi, to try it out here. The College lay outside the area to be protected by the five-year plan, so the expenses of clearing, labour, tools, etc., were to be covered by the College authorities, while the Department would provide supervision and assess the amount of control achieved.

Two independent methods, traps and fly-boys, were used to assess the fly reduction in the experimental clearing, which was to be made on the valley of the Wiwi stream, flowing south quite close to the lecture halls, offices, and dormitories. The incidence of tsetse in the Wiwi valley, as measured by traps and by fly-boy catches, was compared with that, assessed in the same way, of

TABLE IV.
Fly records. Wiwi stream and control rounds.

Month 51/52	Control		Clearing		Reduction %		Rainfall inches	R.H. %	Progress of clearing
	Traps	F-boys	Traps	F-boys	Traps	F-boys			
July	—	55	—	121	—	— 120*	9.41	90	Uncleared
Aug.	89	37	51	75	42	— 103*	2.08	90	Started
Sept.	54	43	29	16	46	61	6.49	92	$\frac{1}{2}$
Oct.	29	59	16	5	45	92	4.89	90	$\frac{3}{4}$
Nov.	12	55	2	9	83	84	4.10	87	9/10
Dec.	21	82	5	6	76	93	0.00	93	Finished
Jan.	64	73	3	3	95	96	0.15	85	
Feb.	76	85	7	5	91	94	3.16	80	
March	71	71	5	8	93	89	5.84	85	
April	99	120	10	14	90	88	6.25	85	
May	178	141	17	15	91	89	8.11	87	
June	194	202	20	22	90	89	9.19	89	
July	96	87	19	7	80	92	9.80	91	

* More flies were recorded from Wiwi than from the control. Fly-boy catches standardised to 10 boy-days per month. The same number of traps in the control as at Wiwi, five in each place.

another comparable valley, which was left in its original condition. The vegetation association in both valleys was originally very similar, in fact the Wiwi was a tributary of the stream selected as control.

It was expected from the start that, on account of the very close proximity of the vast habitat of *Glossina* and the high humidity prevailing, eradication would not be absolute. This indeed proved to be the case, but the reduction achieved was such that clearing was warranted even in conditions of this difficult nature.

Table IV shows the records of fly catches, by the two methods, before the commencement, during, and after the clearing of the Wiwi stream valley was completed, together with the catches recorded from the control stream. It will be seen that the reduction after clearing was about 90 per cent., as assessed both by traps and the fly-boy round. It is interesting that these results show similar rates of control to those obtained by Morris (1946) with protective clearings in savannah.

There may be some relation between the rainfall and the numbers of flies recorded. It has been found on the whole that, the higher the monthly rainfall, the more flies are captured during that month.

The success achieved by clearing the Wiwi stream valley showed that a remarkably high rate of control can be obtained even close to the high forest. It was decided, therefore, to continue clearing other valleys in this area and clearing of a valley south of the staff bungalows is now in progress.

Cost of Clearing and its Value.

During the 1951-52 financial year the cost of labour for clearing 290 acres of bush was £5,006. The cost varied from £25 to £14 10s. per acre, depending on the thickness of bush, state of ground, time of the year, and the amount of supervision.

It is not possible, as yet, to assess the direct benefits by showing the reduction of trypanosomiasis incidence as the clearings made to date in Kumasi are too recent. The preventative value, no doubt great, can only be inferred, and does not lend itself to precise figures as yet.

One of the most encouraging and important features of this work has been the way in which the land has been utilised as soon as it has been cleared. The demand for small plots of land for cultivation, especially among the Northern labourers settled in Kumasi, is so great that the very suitable land along the valleys is quickly being taken up for farms and gardens. A planned scheme for the distribution of the land as allotments has been under consideration, as well as the utilisation of parts of the Dechem valley as a green belt or pleasure park. The Kedjetia valley has already been turned into a racecourse. Developments of this kind ensure that the clearings will be maintained, a proceeding that would otherwise be costly, and provide an added justification for the expenditure of money and effort on the original clearings.

Summary.

Kumasi is a rapidly expanding township of about 80,000 inhabitants. This has led to an extensive building programme which has followed the ridges in the vicinity of the town, leaving the valleys in between potentially dangerous isolated habitats of *Glossina*. The danger from these isolated habitats is enhanced by the large numbers of itinerant labourers.

The topography of the town and its surroundings is hilly; the vegetation is of semi-deciduous rain forest type, but with very thick secondary bush along most of the valleys in which the farms had been abandoned.

The climate is remarkably equable with the rainfall showing periodicity but the percentage relative humidity is high and even throughout the year.

The experimental clearing of an isolated habitat of *Glossina* in the Dechem valley was commenced in July 1950 and finished in March 1951. The clearing was of a discriminative nature, with cutting, stumping and burning of a strip of bush up to 20 ft. high and 250–300 yards wide along the stream, and a high reduction of fly population was achieved. This reduction was such that further clearings were planned.

The reduction in fly population was assessed from fly-boy catches and from trap catches. These records showed that rainfall affected the catches.

At the time of writing, about a sixth of the protective clearings planned around Kumasi have been completed, the fly population being reduced between about 80 per cent. and complete eradication.

Kedjetia, one of the most dangerous valleys on account of its location, was cleared with such success that a complete eradication of *Glossina* was achieved.

The clearing of the Dechem valley was followed by that of the Aboabo valley, thus rendering the eastern side of Kumasi practically vector free. The western and south-western sides of Kumasi will be protected by clearing now in progress on the Danyami stream.

Clearing at the Regional College of Technology was carried out successfully and despite the fact that the College area is surrounded by a vast habitat of the fly, the reduction achieved, as assessed from fly-boy and trap records, was about 90 per cent.

It has been calculated that the cost of clearing one acre has been between £14 10s. and £25.

The value of the clearing cannot yet be correlated with the decrease in cases of trypanosomiasis as the work is too recent, but if one can judge by tsetse reduction it will have preventative and protective value. The utilisation of the cleared valleys for farming and amenities by the local population will not only help to avoid costly maintenance but provides an added justification for clearing.

Acknowledgements.

It is with great pleasure that the writer takes this opportunity of acknowledging the stimulating criticism, advice and help given to him by Dr. K. R. S. Morris, both in initiating the writer in tsetse control work, and throughout the writing of this paper.

References.

- COOKE, W. E., GREGG, A. L. & MANSON-BAHR, P. H. (1937). Recent experiences of mild or symptomless infections with *Trypanosoma gambiense* from the Gold Coast and Nigeria.—Trans. R. Soc. trop. Med. Hyg., **30**, pp. 461–466.
- MORRIS, K. R. S. (1946). The control of trypanosomiasis by entomological means.—Bull. ent. Res., **37**, pp. 201–250.
- MORRIS, K. R. S. (1951). The ecology of epidemic sleeping sickness. I. The significance of location.—Bull. ent. Res., **42**, pp. 427–443.
- MORRIS, K. R. S. & MORRIS, M. G. (1949). The use of traps against tsetse in West Africa.—Bull. ent. Res., **39**, pp. 491–528.
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THE CONTROL OF *GLOSSINA PALPALIS FUSCIPES* NEWSTEAD IN KENYA COLONY.

By S. G. WILSON.

Chief Field Zoologist, Veterinary Department, Kenya.*

P.E

(PLATE XIII.)

Glossina palpalis (R.-D.) is widely distributed in all districts in Kenya bordering on Lake Victoria. It is firmly established along the actual lake shore, both in Central and South Nyanza districts, at an altitude of 3,500 ft. above sea level, and it also extends many miles inland along the main rivers to an altitude of at least 4,700 ft., breeding taking place up to 4,500 ft. above sea level.

In the past, the chief methods adopted to control this species of tsetse in Kenya have either been by hand-catching or by the total or discriminate bush-clearing of the river banks. In this paper these methods are reviewed and the results are given following the spraying of the riverine peripheral vegetation on the Nyando, Mbogo, and Ainamotua rivers with DDT preparations.

Hand-Catching Experiments.

Symes and Vane (1937) eradicated *G. palpalis* by hand-catching from the Nthiwa, Mirogi and Pala streams, tributaries of the Kuja river in South Nyanza district. The original fly-concentration on these streams was of low to medium density and the area was reasonably well isolated. Glasgow and Duffy (1947) eradicated fly by this same method from two isolated blocks on the Sari river and concluded that "hand-catching was the quickest and cheapest known method of reclaiming streams of this type from *G. palpalis*".

Some years later, the same authors (Glasgow & Duffy, 1951) found it impracticable to eradicate *G. palpalis* from an isolated heavily infested block on the main Kuja river. The chief reason for failure was that flies could cross complete riverine clearings of $3\frac{1}{2}$ miles and in a few instances flies were known to travel along the river from 10 to 13 miles. Complete isolation of convenient riverine stretches in which hand-catching could be practised was not therefore a practical proposition on a large heavily infested river.

The Veterinary Department in Kenya has experimented with hand-catching in several areas; more particularly in an isolated pocket at Kitere in South Nyanza, where it was judged a success, and on a tributary of the Yala river in Central Nyanza, when it proved expensive. Hand-catching, therefore, has a limited application in the control of *G. palpalis* in Kenya. It cannot be applied to the extensive lake-shore infestations, to infestations on islands, or to heavy infestations along the main rivers.

Total and Discriminative Bush-Clearing of River Banks.

Within recent years clearing of bush from river banks has been carried out on a relatively large scale along three main rivers, the Yala river, the Magori river, and the Nyando river and its tributaries.

* Now Director of Veterinary Services, North Region, Nigeria.

Yala River.

During 1948-49 the riverine bush along the upper reaches of the Yala river was cleared from some distance below Yala station to the Abom bridge, about $13\frac{1}{2}$ miles, i.e., blocks A, B, C, D and E being cleared (fig. 1). The first four

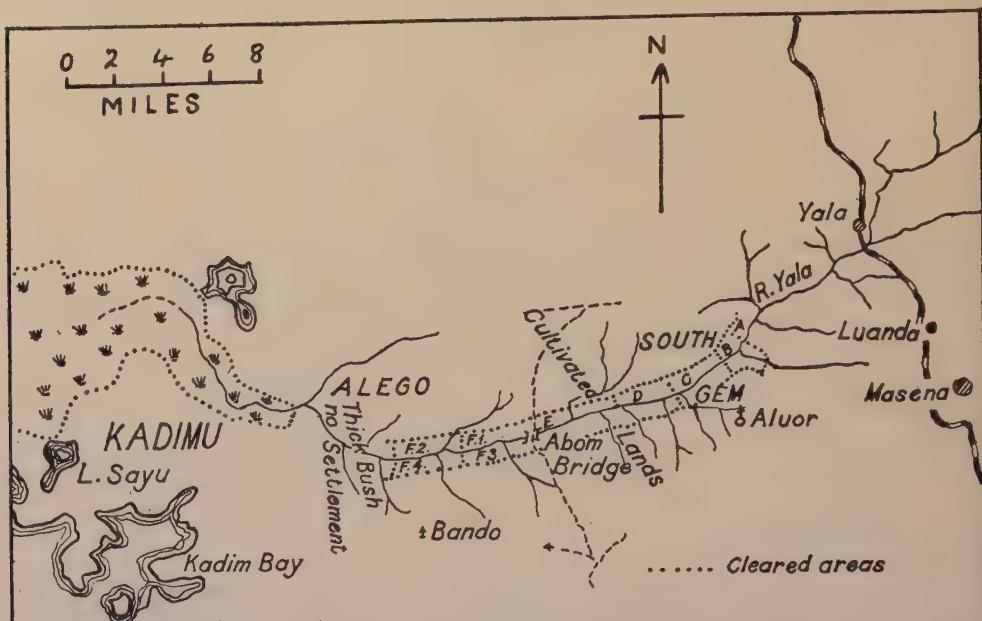


Fig. 1.—Yala river in Central Nyanza, Kenya, showing blocks A to F where riverine bush-clearing has been carried out.

TABLE I.

Total *G. palpalis* caught each month from August 1948 to December 1949 as a result of weekly patrols along blocks A to E on Yala river.

Month	Blocks				
	A	B	C	D	E
August 1948	27	143	—	187	—
September	.	56	15	302	—
October	.	48	13	298	—
November	.	45	7	386	—
December	.	32	1	288	—
January 1949	.	36	.	154	—
February	.	30	.	85	—
March	.	9	.	86	—
April	.	13	.	128	—
May	1	8	.	116	488
June	.	6	.	146	535
July	.	8	.	146	756
August	.	8	.	55	975
September	.	5	.	51	448
October	.	.	.	37	478
November	.	4	.	34	239
December	.	3	.	9	123

blocks carried very light riverine bush and the banks were totally cleared but the river banks along block E were heavily bushed and were discriminately cleared, 10 to 15 of the large trees being left per acre. The results of this clearing on the fly-population up to the end of 1949 are shown in Table I. *G. palpalis* has been eliminated from blocks A and C where in any case the original fly-population had been small. Only a few fly remained in blocks B and D but in block E a considerable fly-population remained.

It was therefore realised early in 1950 that two further operations were necessary in order to eradicate fly from block E and to render the river crossing safe at Abom bridge. The number of trees and shrubs left in block E by the original discriminative clearing would have to be further reduced and certain tributaries would also require clearing. Further, in order to prevent dispersal of flies into block E from the heavy infestations still remaining west of Abom bridge, bush-clearings would have to be carried still further downstream.

Following the additional clearings carried out in block E during the first four months of 1950 the number of *G. palpalis* caught fell from 137 flies in January 1950 to 10 in December 1950 but during 1951 flies continued to be caught at the rate of 18 to 26 per month.

In January 1950, four fly patrol-routes were marked out downstream from Abom bridge in block F, patrol F1 and F3 working downstream from Abom bridge on each bank for $3\frac{1}{2}$ miles, while F2 and F4 started at the end of routes F1 and F3 and worked further downstream for an additional distance of $3\frac{1}{2}$ miles (fig. 1). The routes were traversed once weekly by two fly-boys, all flies being caught which either came to the screen, attacked the fly-boys or could be caught from nearby vegetation.

Bush-clearing was carried out from August to December 1950 along the river banks following fly patrol-routes F1 and F3, while patrol-routes F2 and F4 were left untouched. The results of bush-clearing on the total flies caught along F1 and F3 as compared with the patrol-routes F2 and F4 in untouched bush is shown in Table II.

TABLE II.

Total *G. palpalis* caught each month on the Yala river along four patrol-routes downstream from Abom bridge. January 1950–June 1951.

Patrols	Riverine bush cleared August–December 1950		No bush-clearing carried out	
	F 1	F 3	F 2	F 4
January 1950	264	248	269	268
February	115	116	120	144
March	111	154	118	178
April	115	136	120	166
May	191	177	218	168
June	162	151	199	182
July	139	157	210	207
August	143	160	182	190
September	134	109	213	200
October	97	83	196	176
November	61	61	209	214
December	10	17	186	199
January 1951	4	4	185	190
February	4	3	199	173
March	3	7	266	267
April	5	4	232	217
May	11	21	220	203
June	20	21	194	208

It is evident that *G. palpalis* was almost completely eradicated from the area covered by patrol-routes F1 and F3. The increased catches however during May and June 1951 show that, as might be expected, fly is still dispersing into the area from the adjacent heavily infested areas covered by patrol-routes F2 and F4. Since human settlement in this area is still not possible, regeneration of the riverine bank with increased *G. palpalis* infestation may be expected.

The cost of clearing the 17 miles of river bank and approximately 3 miles of tributaries over the years 1948-1950 was about £4,000, or £200 per mile.

Magori River.

The Magori river is infested with various species of tsetse along its entire course of 60 miles from its origin in Masailand to its junction with the Kuja river near Lake Victoria. Along its upper reaches *G. pallidipes* Aust., *G. brevipalpis* Newst. and *G. fuscipleuris* Aust. occur, while downstream from the Magori trading centre, *G. palpalis* is the predominant species along the main river, whilst *G. pallidipes* occurs along the large tributaries.

Prior to 1949, the only clearings made on the Magori river system were three anti-human sleeping sickness clearings at road crossings—one of 2,000 yards long at the main Suna road bridge at Magori and two of approximately 1,500 yards each on road crossings near the junction of the Magori river with the Kuja river at the Macalder Mines. It was decided in 1949 to join these clearings and the first part of the scheme was to clear the 10-mile stretch of the main river west of Magori, starting at the Suna road bridge and working downstream.

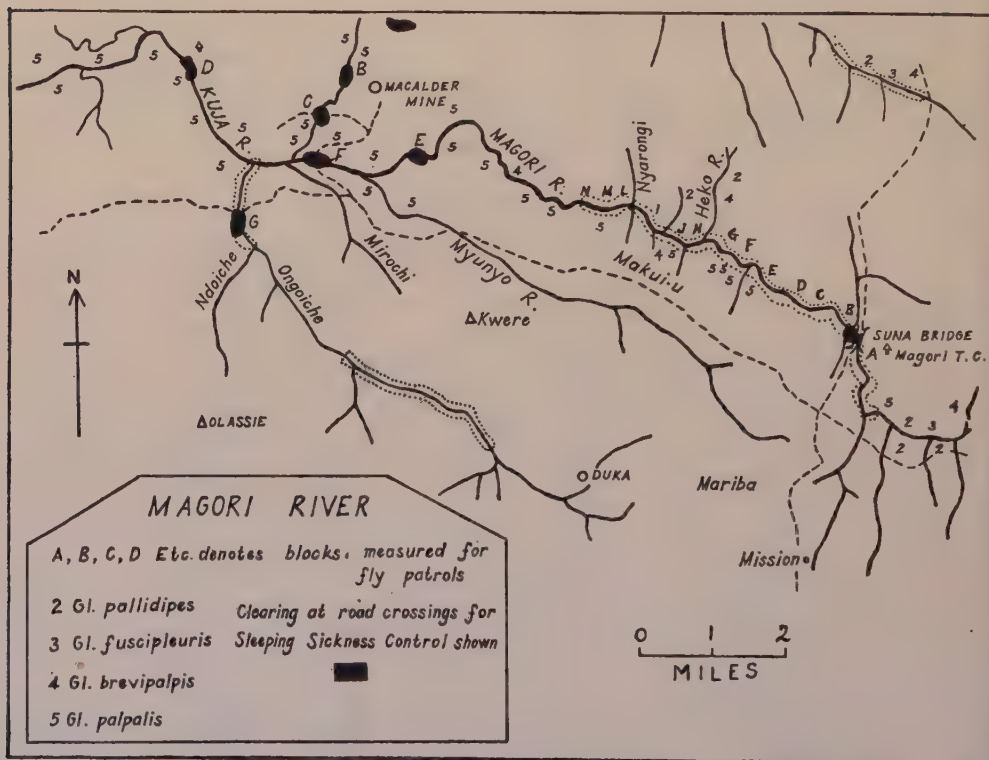


Fig. 2.—Magori river in South Nyanza, Kenya, showing blocks A to N where riverine bush-clearing was carried out.

The 10 miles of main river were arbitrarily divided into 13 blocks labelled A to N (fig. 2). Discriminative clearing, leaving 10 to 15 of the taller trees per acre was started in June 1949 from two camps, one in block A and one in block L near the Nyarongi tributary. Fly-patrols by African fly-boys using dark screens were also started along most blocks, each block being patrolled once weekly.

By the end of December 1949 the bush along the main river stretch of 10 miles had been cleared and a considerable reduction in fly-catches had occurred. It was, however, obvious that too many trees had been left along the cleared river banks and *G. palpalis* were dispersing upstream from the heavy infestations below into block M. Also, two full pupae of *G. palpalis* were found in the cleared area in block N three months after clearing and fly-breeding was also still taking place in block G.

Further clearing was therefore carried out in 1950 along the main river and along 9 small tributaries and cut bush was piled and burned. A summary of the results of catches of *Glossina palpalis* during June and December of each year in each block on the main Magori river is given in Table III.

TABLE III.

Total number of *G. palpalis* caught in each block on Magori river in 5 selected months between June 1949 and June 1951.

Block	1949		1950		1951
	June	December	June	December	June
A	49	38	3	0	0
B	86	10	0	0	0
C	446	31	1	0	0
D	822	110	12	0	1
E	Not patrolled	262	10	0	0
F	"	145	16	2	0
G	"	1033	107	1	2
H	1412	231	124	1	1
J	238	132	95	1	1
K	115	132	25	15	2
L	1052	205	48	1	11
M	1074	198	60	9	12
N	718	579	387	117	145

In addition to the reduction of *palpalis* caught along the main river the extra bush-clearing had practically eradicated *G. pallidipes* from the major tributaries.

The cost of this scheme over the three years 1949 to 1951 was £5,307 and during this time 10 miles of main river course and 3 miles of tributaries had been cleared. The cost therefore of clearing was approximately £350 per mile. Further progress westwards with this scheme will be difficult because the riverine area is infertile and without human settlement the future maintenance of clearings will be expensive.

Nyando River System.

The Nyando river and its main tributaries, the Gundas, Ainamotua, and Mbogo rivers, were surveyed for tsetse infestations during December 1948 to February 1949. All these rivers were found to be infested with *G. palpalis* in varying concentrations. This tsetse survey coincided with a serious outbreak of human sleeping sickness in the African population around Kibigori so the importance of tsetse eradication in this area gained in emphasis.

The original scheme suggested was to clear the bush from the banks of all the infested streams, the estimated total clearings necessary being about 80 miles. The time allowed for the scheme was two years (1949–1950) and the total cost estimated at £5,963.

The main rivers were divided arbitrarily into blocks to simplify the fly-patrol system, each block at first varying in distance from 5,000 to 7,000 yards long but later blocks along the Ainamotua were each 6,000 yards long. Each block was patrolled once weekly by two fly-boys with a dark cloth screen. Blocks A, B, C and D were on the main Nyando river, block E, G and W were on the Mbogo river below the confluence of the Ainamotua river, while T, P, Q, R and S were on the upper stretches of the Mbogo, blocks F, H and I were on the Gundas river and blocks U, V, J, K, KIP, L, M, N, O, Y and Z were on the Ainamotua river system (fig. 3).

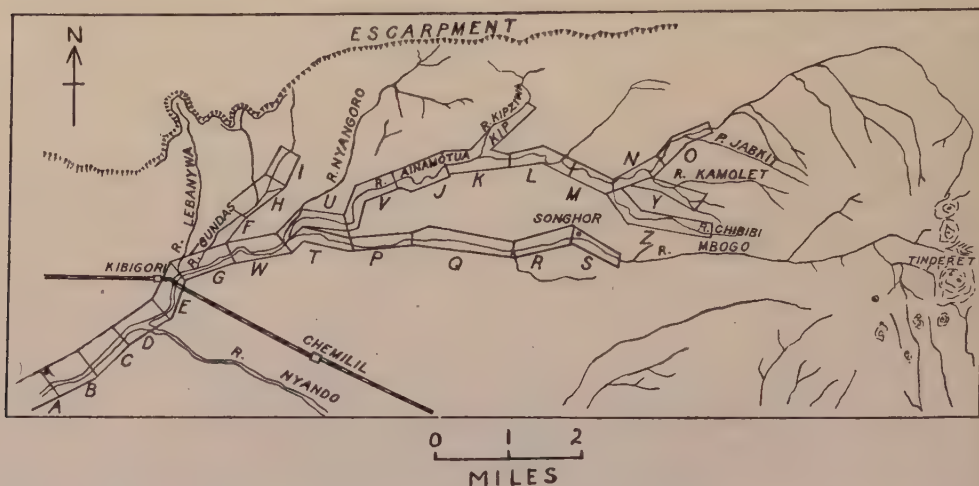


Fig. 3.—The Mbogo, Ainamotua and Gundas tributaries of the Nyando river system in Central Nyanza, Kenya, where bush-clearing and spraying operations were carried out against *G. palpalis*.

Bush-clearing commenced in May 1949 and by the end of that year blocks A, B, C, D, E, G and W on the Nyando-Mbogo rivers and blocks F, H and I on the Gundas river had been cleared. The type of clearing was discriminative, but when the position was reviewed early in 1950 the standard of work was so poor that burning was impossible. Many of the felled trees were left unlopped and lying in the river bed and too many trees and shrubs had been left uncut. No regular system of patrols had been carried out and fly records were not available to show what progress had been achieved but it was evident that most of the area was still heavily infested with *G. palpalis*. The only exception was along blocks A, B and C where the Nyando river banks were open and cultivated, and the original infestation of fly had been light. The work during the year 1949 had cost £2,030 and as 20 miles of river had been covered the cost was approximately £100 per mile.

The major task in 1950 was to re-slash most of this area which had been cleared in 1949 and to cut and pile the felled trees ready for burning. Also, in blocks E, F and G, clumps of forest harbouring tsetse up to 200 yards away from the river banks were cleared. Some wholesale clearing was also carried out in the distal ends of blocks U and T. Regular weekly fly-patrols on defined routes along the river banks were established.

By December 1950, fly had apparently been eliminated from block D on the Nyando river and on the Mbogo river. The best results were on the Gundas river (fig. 4) where *G. palpalis* disappeared after an additional burn along the river banks early in 1951.

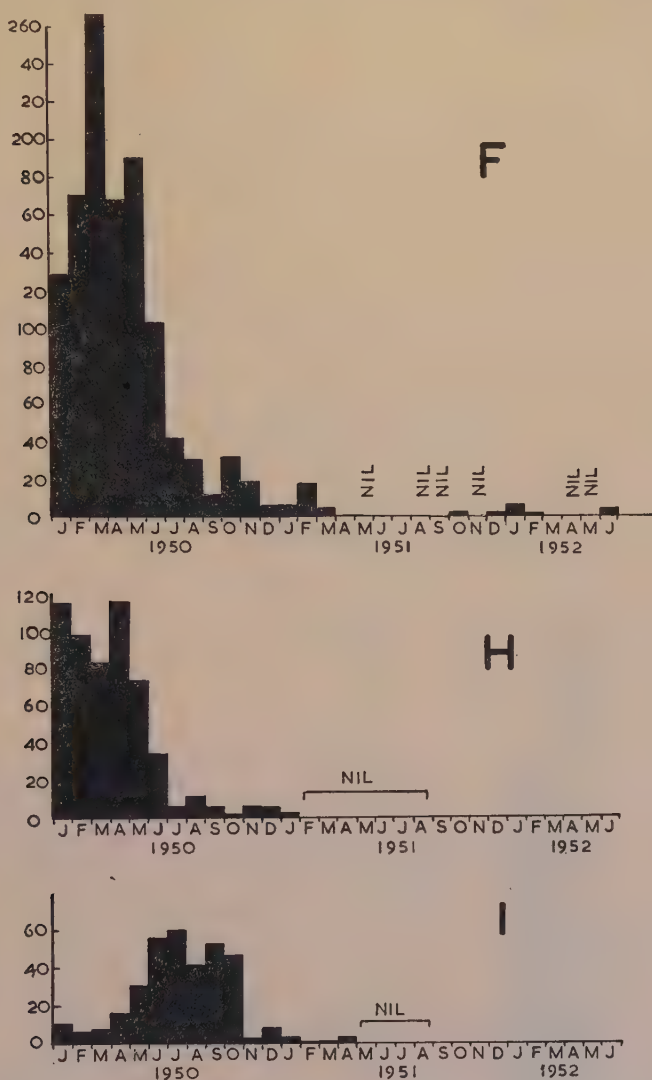


Fig. 4.—Total *G. palpalis* caught each month by 4 weekly patrols in blocks F, H and I on the Gundas river.

The cost of this second clearing in 1950 over approximately 12 miles of river banks was £1,200 or £100 per mile. The total cost therefore for the clearing done in both 1949 and 1950 over most of the blocks was £200 per mile. The cost of continuing this method of eradicating *G. palpalis* was obviously prohibitive. In addition, it was becoming increasingly difficult to recruit an adequate labour force. An alternative method had therefore to be found if the scheme was to progress as originally intended.

Control of *G. palpalis* by DDT Sprays.

The Colonial Insecticides Unit, Entebbe, had shown that, by spraying only the vegetation along the water's edge, the density of *G. palpalis* on an island was considerably reduced (Woodcock, 1949). The Unit was anxious to apply this method to a riverine area and offered their expert assistance and financial help to further our efforts on the Nyando river system.

Mbogo River.

Blocks T, P, Q, R and S on the Mbogo river (fig. 3) were chosen for the preliminary spraying trials as they were heavily infested with *G. palpalis* and bordered the farms on which most of the cases of human sleeping sickness had occurred. It was also considered that this portion of the river system could be relatively easily isolated from existing infestations in block U on the Ainamotua river and from block W and G on the lower reaches of the Mbogo river. Bush along the banks in blocks G and W and for 500 yards upstream in block U had already been cleared. As will be noted later, however, the isolation of block T was never satisfactory.

The length of the Mbogo river covering blocks T to S was approximately 20 miles. It was decided in the first instance to apply four complete applications of spray at 14-day intervals to the fringing riverine vegetation along the entire length of these blocks. The method of spraying was for two labourers equipped with knapsack pressure sprayers to walk along the river bed and spray all vegetation within their reach. A team of three boys was able to spray $2\frac{1}{2}$ to 3 miles of river in one day, spraying both banks. Sprayers at work along the river bank in block T are shown in Plate XIII.

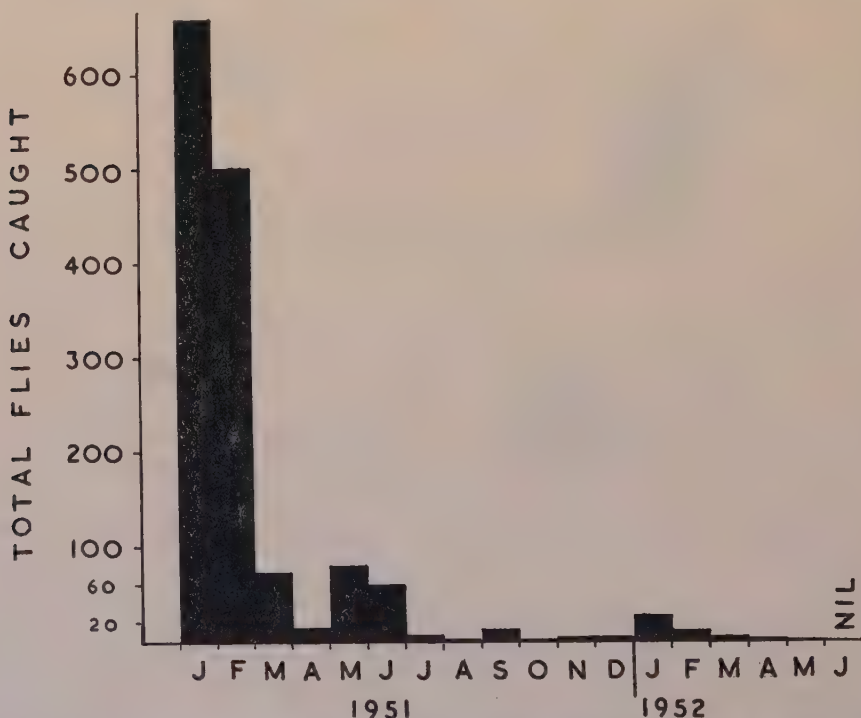


Fig. 5.—Total *G. palpalis* caught each month by 4 weekly patrols in blocks T, P, Q, R and S on the Mbogo river.

The vegetation along the river banks varied greatly in depth and in composition. In blocks T and P the predominant vegetation was the fringing tall grasses, sedges, small shrubs and climbers which grew along the river banks. Tall tree cover occurred only in isolated spots. In block Q, where the concentration of *G. palpalis* was highest and breeding prevalent, there were considerable stands of tall trees such as *Bridelia micrantha*, *Vitex cuneata*, *Albizia zygia*, *Ficus* spp., *Teclea nobilis* and *Stereospermum kunthianum*. Underneath these trees small shrubs such as *Scutia buxifolia*, *Carissa edulis* and *Rhus natalensis* occurred while along the immediate river banks small palms, *Phoenix reclinata*, and shrubs of the *Dombeya* and *Hibiscus* spp., together with *Phragmites*, *Echinochloa* spp., and sedges grew in profusion. In block R, however, considerable stretches of the river banks were bare of vegetation and required little attention.

This riverine belt of vegetation was usually, however, extremely narrow in all parts as, only a few yards away from the river, a *Combretum-Bauhinia*-open grassland formation prevailed.

The material used for spraying in the first instance was a 50 per cent. DDT paste dispensed in 11-lb. tins. Each tin of this paste was carefully mixed with 10 gallons of water on the river bank at the time of spraying so that the final spraying mixture contained 5 per cent. DDT, approximately. Later, an emulsion containing 50 per cent. DDT and one containing 15 to 17 per cent. DDT (calculated as 15 per cent.) were used, but they were more difficult to transport over difficult terrain.

Four complete applications to 20 miles of river banks were made between February and May 1951 and 165 tins of 50 per cent. DDT paste, in addition to 40 gallons of a 50 per cent. DDT emulsion, were used. A total, therefore, of 1,107 lb. of DDT was applied to 80 miles of fringing riverine vegetation, an approximate average of 13.8 lb. per mile on each application or a total of 55.2 lb. per mile during the four applications. If it could be assumed that 2 yards of vegetation on each bank were sprayed and that the vegetation was uniform over the whole

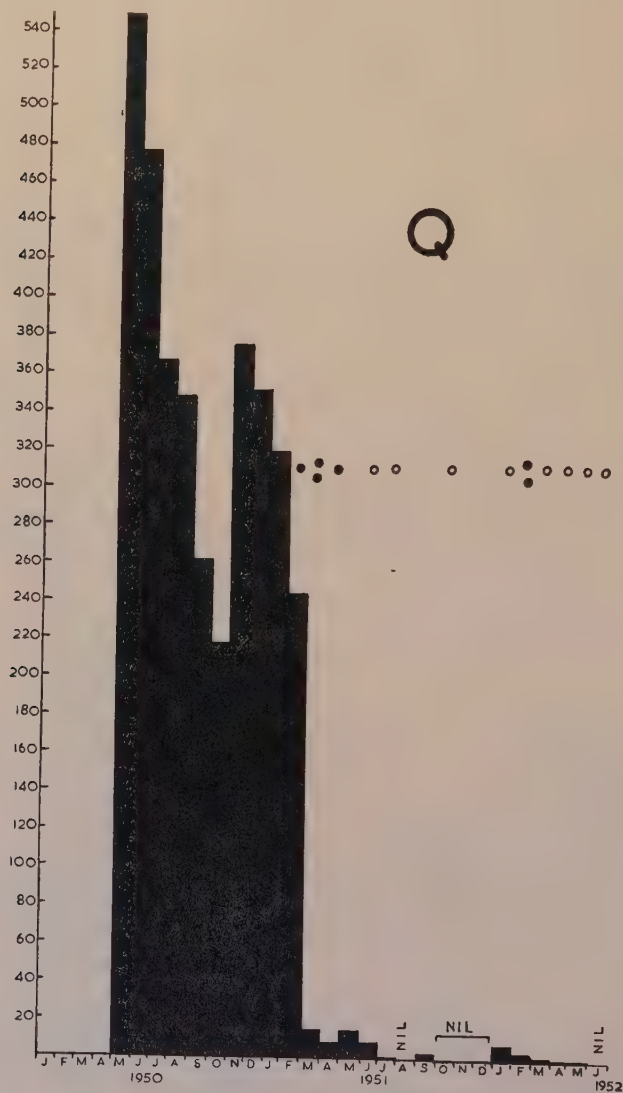
TABLE IV.

Total *G. palpalis* caught and quantities of DDT used on blocks T, P, Q, R and S on the Mbogo river.

Month	Total <i>G. palpalis</i> caught	Quantities DDT preparation used	lb. DDT	Rainfall
January 1951	660	Nil	Nil	2.6
February	502	374 lb. paste	187	3.24
March	77	1078 " "	539	3.44
April	17	363 " " +20 gals. 50% emulsion	281.5	11.60
May	80	20 gals. 50% emulsion	100	13.89
June	60	40 " "	200	2.83
July	7	7 " 15% "	10.5	2.82
August	2	Nil	—	4.72
September	12	Nil	—	3.01
October	1	13 gals. 15% emulsion	19.5	3.76
November	4	Nil	—	13.78
December	4	33 lb. paste	16.5	6.28
January 1952	27	88 lb. paste	44	0.5
February	11	264 " "	132	4.61
March	5	44 " " +16 gals. 15% emulsion	46	3.47
April	2	10 gals. 15% emulsion	15	15.55
May	1	8 " " "	12	12.02
June	0	8 " " "	12	1.95

area the rate of application would be 10 lb. DDT per acre on each application. The vegetation, and therefore the spraying rate, was however far from uniform and on the heavily infested foci in blocks P and Q the rate of application was heavier and was estimated as being between 15 to 20 lb. DDT per acre.

The residual effect of the spray on the vegetation after 14 days exposure was excellent. In the first experiment carried out in February when rainfall was low, 20 *G. palpalis* were exposed for 20 seconds on vegetation collected 14 days after spraying. Twelve of the exposed flies were dead 18 hours later while



only 2 out of 20 control flies had died. In a similar experiment in March, 30 out of 31 exposed flies died within 16 hours while only 1 control fly died. The residual effect therefore of the DDT deposit on vegetation at this rate of spraying in dry weather after 14 days exposure could be considered satisfactory.

Following the spraying in February, March and April the total number of tsetse caught in all five blocks on the Mbogo river during weekly patrols along each bank dropped from 660 flies caught in January to 17 in April (fig. 5 and Table IV). The dramatic nature of this fall in numbers during February and March 1951 is well shown by the returns from block Q, which was the most heavily infested area on the Mbogo river (fig. 6). The heavy rains in April and May had however unfortunate effects. Owing to the swollen state of the rivers satisfactory spraying of the fringing vegetation on the river banks was often impossible, while the heavy downpours of rain soon washed the DDT deposit from the leaves of the vegetation. The residual effect of the spraying was therefore shortened and the rise in fly catches in May 1951 was not entirely unexpected.

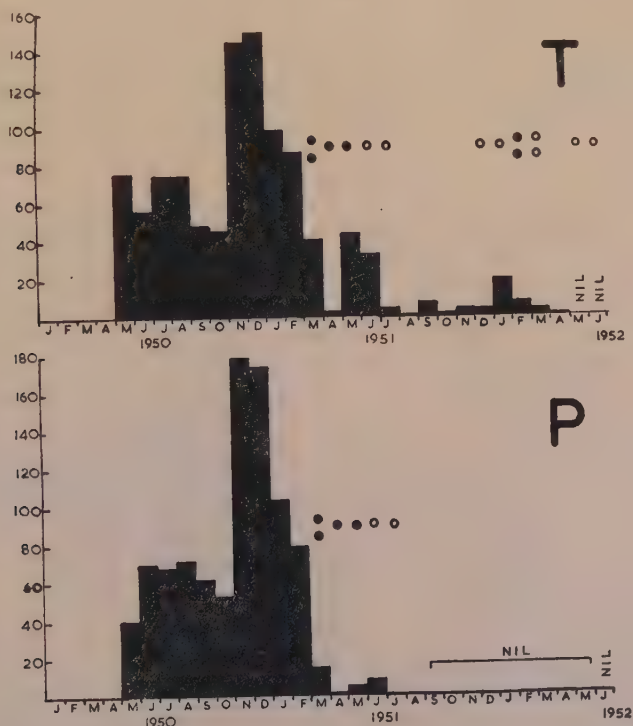


Fig. 7.—Total *G. palpalis* caught each month by 4 weekly patrols in blocks T and P on the Mbogo river before and after spraying with DDT.

An examination of the fly records from each block however showed that the rise in May and June 1951 shown in the gross returns from all blocks (fig. 5) had occurred chiefly in block T. A comparison of the number of flies caught in the two adjoining blocks T and P illustrates this point (fig. 7). Both blocks up to the end of July 1951 had received identical spraying treatments. During May and June there was a slight increase in the numbers caught in P but they were soon eradicated. In block T the increase in the numbers caught during May

and June was serious and although reduced after the July spraying they did not quickly disappear.

The behaviour of the fly in block T could not be due solely to extensive breeding in this area since the vegetation in block T is less suited for pupal sites than, for instance, that of block Q. In the region of block T, the Ainamotua river flows within a few hundred yards of the Mbogo river for a distance of almost a mile, and cattle were constantly moving across from drinking places in block U to drinking places and river crossings in block T (fig. 3). The movement of *G. palpalis* from one block to the other was later proved when flies marked and released in block U were recovered in block T.

It was therefore decided in June 1951 that, in addition to spraying in blocks T, P, Q, R and S on the Mbogo river, spraying should also be carried out at the same time in block U on the Ainamotua. As will be noted later this spraying was soon extended to include the entire Ainamotua river. At the same time the system of spraying the fringing vegetation along the entire length of the blocks was largely abandoned in favour of spraying smaller localised areas where fly were continuing to be caught. This type of spraying is referred to as partial spraying.

The amounts of DDT applied each month on the Mbogo blocks in relation to the number of flies caught and the monthly rainfall are shown in Table IV, while the distances sprayed and rates of DDT per acre from June 1951 to June 1952 are given in Table V.

TABLE V.

Distances sprayed and the amounts of DDT applied along the Mbogo river from June 1951 to June 1952.

Month	Blocks sprayed	Distance sprayed in yards	lb. DDT used	Rate DDT per acre in lb.
June 1951	T.P.Q.R.S.	17,000	200.0	13.3
July	T.P.Q.	5,000	10.5	2.6
October	Q.R.	5,000	19.5	4.5
December	T.	1,000	16.5	19.8
January 1952	T.Q.	6,000	44.0	8.8
February	T.Q.	18,000	132.0	8.8
March	T.Q.	5,000	46.0	11.0
April	Q.	2,000	15.0	9.0
May	T.Q.	3,000	12.0	5.0
June	T.Q.	4,000	12.0	4.0

Space does not permit giving the results from each block but fly in blocks R and S on the headwaters of the Mbogo behaved very similarly to those in block P (fig. 7) and the last fly was caught in June 1951. Progress in block Q (fig. 6) was slower and fly persisted, in spite of fairly extensive spraying, until May 1952.

The elimination of fly in block T depended, as stated above, on the simultaneous control in block U and to a lesser extent in block W. Fly was brought under control in these two latter blocks by spraying, and the control in block T, following spraying from January 1952 onwards, was excellent and no *G. palpalis* has been caught in T since April 1952.

During June 1952, 17 months after the spraying operations had commenced, no fly was caught in blocks W, T, P, Q, R and S on the Mbogo and only one was caught on the adjacent block U on the Ainamotua. During this period, from

February 1951 to June 1952, 204 11-lb. tins of 50 per cent. DDT paste, 80 gallons of a 50 per cent. emulsion and 62 gallons of a 15 per cent. emulsion had been used on blocks T to S (Table V). The 204 tins of paste cost locally 45s. per tin or a total amount of £459; the 80 gallons of 50 per cent. emulsion was an experimental lot but is charged at 30s. per gallon or a total of £120; while 49 gallons of the 15 per cent. DDT emulsion cost 14s. a gallon and 13 gallons cost 22s. per gallon or a total of £48 6s. The total cost therefore of the spraying materials for the 20 miles of river was approximately £630. The cost of the spraying team of 3 boys for 17 months was £150 with an extra £50 to cover the cost of pressure sprayers and incidentals. The total cost was therefore £830 or £42, approximately, per mile of river. This compares very favourably with the current costs of bush-clearing which are never below £200 per mile.

Ainamotua River.

The initial spraying of the fringing vegetation in block U of the Ainamotua river, as noted above, was carried out in June 1951 in order to make possible the eradication of *G. palpalis* in block T of the Mbogo. Spraying was gradually extended to the other blocks upstream on the Ainamotua from August 1951 onwards.

The Ainamotua is an extremely difficult river to work as the banks are steep and heavily overgrown with *Phragmites*, *Echinochloa* spp. and other tall grasses and sedges. Spraying operations were therefore slow and difficult to carry out.

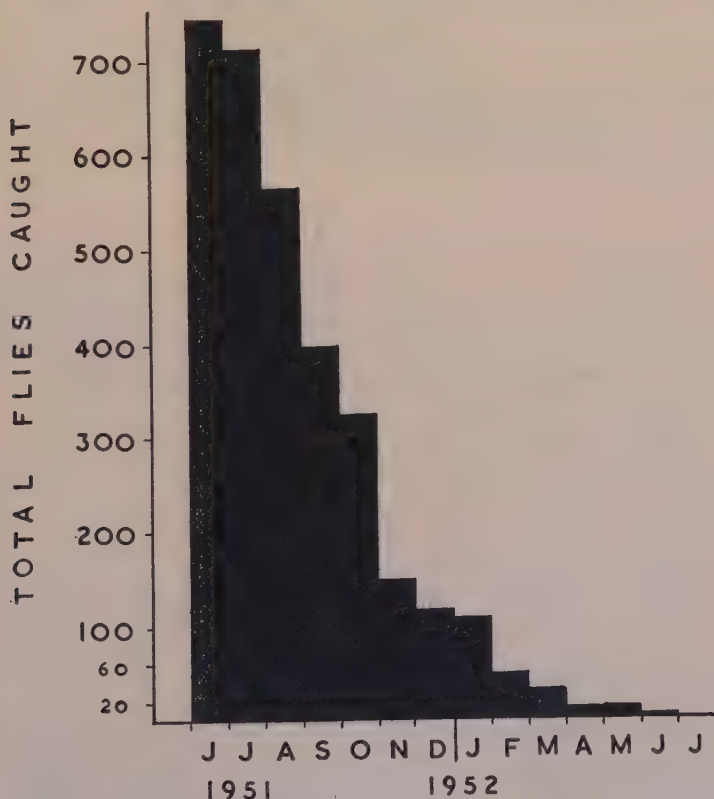


Fig. 8.—Total *G. palpalis* caught each month by 4 weekly patrols in blocks U, V, J, K, L, M, and KIP on the Ainamotua river.

Also, in the latter half of 1951 it was difficult to obtain adequate supplies of suitable DDT preparations. In the first instance, therefore, arrangements were made to spray blocks U, V, J, K, L, M, and later block KIP on a tributary was included. The decrease of *G. palpalis* on these 7 blocks from June 1951 to June 1952 is shown in fig. 8, the number of flies caught decreasing from 746 in June 1951 to 5 in June 1952.

The initial decrease in the number of flies caught along these blocks on the Ainamotua was not so rapid or spectacular as along the Mbogo river (fig. 6). The chief reason for this slow decrease was that, owing to the practical difficulties, the original treatments on the Ainamotua were not so heavy or so frequent as on the Mbogo where 4 complete sprayings along the whole river were applied within an initial period of 2 to 3 months. The whole length of the Ainamotua could not be sprayed in one or even two months, so for some time flies were still moving downstream from the unsprayed blocks in the headwaters of the river.

Spraying in blocks Y and Z on the Kamolet and Chibibi tributaries was initiated in November 1951 and the number of *G. palpalis* caught in these two blocks decreased from 127 flies in November 1951 to 3 flies caught in June 1952.

The two blocks N and O on the headwater of the Ainamotua were sprayed from March 1952 onwards, the number of *G. palpalis* caught here decreasing from 101 in March 1952 to 9 flies in June 1952. Fly-patrols were in operation once weekly on all blocks of the Ainamotua as from August 1951 and during that month the total number of flies caught along the whole length of the river was 929. In June 1952 the total catches along the same distance had fallen to 17 flies.

The total length of the Ainamotua including all tributaries (blocks U to Z) was 65,210 yards or 37 miles approximately. Over this area from June 1951 to June 1952 inclusive 650 gallons of 15 per cent. DDT emulsion and 74 × 11-lb. tins of 50 per cent. DDT paste were used or a total of 1,382 lb. of DDT. This

TABLE VI.

Distance along river sprayed and amounts of DDT applied each month in blocks U and L on the Ainamotua.

Month	Block U				Block L			
	<i>G. palpalis</i> caught each month	Distance sprayed in yds.	lb. DDT applied	lb. DDT per acre	<i>G. palpalis</i> caught each month	Distance sprayed in yds.	lb. DDT applied	lb. DDT per acre
June 1951	160	6,000	45.0	9	105	—	—	—
July	30	4,000	19.5	6	84	—	—	—
August	25	nil	nil	nil	143	—	—	—
September	37	4,000	24.0	7.3	85	6,000	30.0	6.0
October	23	6,000	30.0	6.0	59	6,000	27.0	5.6
November	17	8,000	27.0	4.2	18	6,000	18.0	3.6
December	24	5,000	15.0	3.6	14	3,000	7.5	3.0
January 1952	26	4,000	27.5	8.3	24	4,000	22.0	6.6
February	20	6,000	38.5	7.7	2	2,000	16.5	10.3
March	14	nil	nil	nil	4	nil	nil	nil
April	3	12,000	28.5	2.8	3	2,000	6.0	3.8
May	1	nil	nil	nil	3	5,000	24.0	6.0
June	1	3,000	10.5	4.2	2	2,000	6.0	3.8

would give an overall cover for all spraying of 37.3 lb. DDT per mile. The cost of these materials was £638 which is equivalent to approximately £17 per mile.

The actual rate of application of DDT in lb. per mile or per acre varied greatly along various stretches of the river. This variation in the rate of DDT deposited on the two blocks U and L is shown by Table VI. The estimate of lb. of DDT per acre is calculated on the assumption that 2 yards of vegetation on each river bank was sprayed on every occasion so that 6,000 yards of river would be equivalent to 5 acres approximately. The response to spraying in these two blocks is further shown by fig. 9. The initial decrease in flies caught in block U

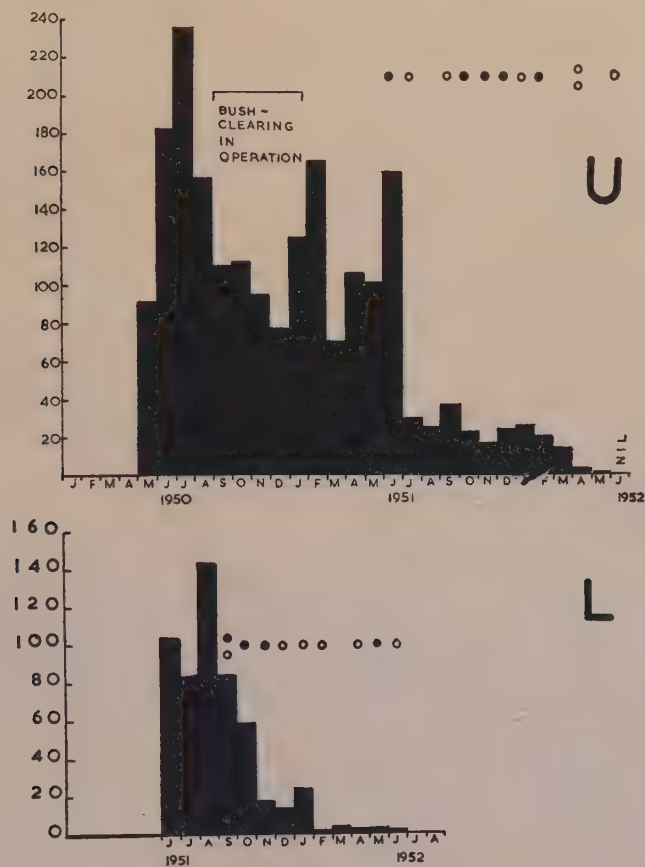


Fig. 9.—Total *G. palpalis* caught each month by 4 weekly patrols in blocks U and L on the Ainamotua river before and after spraying.

in July 1951 was spectacular but thereafter the numbers caught remained surprisingly high until April 1952. By this time the spraying was having its effect on areas further upstream as shown by the low catches in block L from February 1952 onwards. During May and June 1952 the dispersal of flies into block U had practically ceased so eradication became a possibility. For the same reason, after the initial fall in block L in November 1951, final eradication was not possible until flies in blocks M, N and O were controlled.

Discussion of Results.

The hand-catching method of eradicating *G. palpalis* from the larger rivers has not been successful in Kenya. The method of choice therefore for eradicating this tsetse has been by riverine clearings. Results from the Yala and Magori rivers show that this method can be successful even when the original infestations are heavy as in blocks D and E, F1 and F3 on the Yala (Tables I and II) and blocks C to M on the Magori (Table III).

The decrease in fly-catches following clearing is usually gradual and some months elapse before the effects are fully evident. Thus in 1949 when bush clearing along blocks D and E on the Yala river was in full operation fly-catches fell gradually in block D from 154 flies caught in January to 9 in December, while in block E the fall was even slower (Table I). In blocks F1 and F3 the same gradual decrease was evident (Table II).

A disturbing tendency for the fly-catches to increase after bush-clearing operations had ceased was especially evident in the F blocks and in blocks L, M and N on the Magori river. It was evident that the proper isolation of these blocks from contiguous infested areas downstream had not been achieved. Even with the reasonably extensive clearings in blocks E and F on the Yala river, *G. palpalis* can still contact human pedestrians on the Abom bridge.

In order therefore to be permanently successful and ensure eradication something more than mere bush-clearing and burning is necessary. The key to success lies in the establishment of grasslands along the river banks with permanent human settlement. The careful planning that is needed in schemes of this nature is well shown by the work of Morris (1946) in the Gold Coast.

Along the Yala and Magori rivers such human settlements will always be difficult as the areas cleared are relatively narrow, the land often stony and infertile and unsuited for continual cultivation, and in any case the African in these districts is not suffering from any land shortage.

The type of discriminative clearing carried out on all the rivers proved as expensive as total riverine clearings since regeneration had to be checked frequently by re-slashing. Also the success of such clearings depends almost entirely on a successful burn of the bush as soon as possible after cutting. In areas of erratic rainfall where heavy downpours may occur at any season such a rapid burn cannot always be arranged.

The cost of these clearings is expensive, varying from £200 per mile on the Yala to £350 per mile on the Magori river, so that original planning should be such as to ensure success.

For the same reasons, that of expense and the difficulties of maintaining the clearings, the early work on the Nyando, Gundas and Mbogo rivers threatened to be a complete failure. The success therefore with the early DDT sprayings was most encouraging.

The initial fall in the *G. palpalis* population on the 20 miles of the Mbogo river following the first four complete sprayings, was dramatic and surpassed anything previously achieved by bush-clearing. There were some signs of recovery in fly numbers in May and June 1951 and in January 1952, but from July 1951 onwards the density of *G. palpalis* along this 20-mile stretch of river was too low to be a menace to public health. The rapid fall in numbers of *G. palpalis* on the Mbogo river which occurred during February and March, was achieved by the use of 1,452 lb. of 50 per cent. DDT paste at a cost of £297 or approximately £15 per mile. The labour costs also were small and it is much easier to organise a team of 3 to 6 labourers to carry out spraying operations than it is to recruit and maintain a gang of 150 to 200 men to carry out bush-clearing.

The original rate of application of DDT aimed at was to use $3\frac{1}{2} \times 11$ -lb. tins of 50 per cent. paste or 35 gallons, approximately, of a 5 per cent. DDT spray per mile of river. This rate of application (19 lb. DDT per mile) was not achieved if the overall figure of 13.8 lb. per mile or 10 lb. DDT per acre is accepted. The uneven nature of the riverine vegetation is however an important factor, varying from the dense relatively wide thickets in block Q to the open clear banks of parts of block R. It can be accepted therefore that where the density of *G. palpalis* was high the average rate of application during February to April was in the region of 15 to 20 lb. DDT per acre.

In the later phases of this operation when, chiefly, partial spraying was practiced, the rates of application tended to be below 10 lb. per acre, and the delay in eradicating *G. palpalis* from blocks T and Q may have been due to this low rate of application. If a rate of 15 lb. per acre or more had been maintained for several consecutive months fly might have been eradicated at a much earlier date.

Work on the Ainamotua has always been slow and difficult and although block U was first sprayed in June 1951, blocks N and O on the headwaters were not sprayed until March 1952. Also the rate of application of DDT has always tended to be low. Thus the total application by all spraying up to June 1952 of 1,382 lb. DDT or 37.3 lb. per mile compares unfavourably with the rate of the first four applications on the Mbogo when 55.2 lb. DDT per mile was applied. This low rate of application continued throughout the campaign as shown by Table VII. In spite of this however the number of flies caught along the 37

TABLE VII.

Distances sprayed and the amount of DDT applied along the Ainamotua river from June 1951 to June 1952.

Month	Blocks sprayed	Distance sprayed in yards	lb. DDT used	Rate DDT per acre in lb.
June 1951	U	6,000	45	9.0
July	U	4,000	19.5	6.0
August	V.J.K.	18,000	102	7.0
September	U.V.J.K.L.M.	34,000	174	6.1
October	U.V.J.K.L.M.	36,000	150	5.0
November	U.V.J.K.L.M.Y.Z.	40,000	132	4.0
December	U.V.J.K.KIP.L.M.Y.Z.	24,000	124.5	6.3
January 1952	U.V.K.L.M.Y.Z.	18,000	115.5	7.7
February	U.V.J.L.M.Y.Z.	24,000	203.5	10.2
March	V.J.KIP.M.N.O.Y.	18,000	89.5	5.9
April	U.J.L.W.O.Y.	33,000	106.5	3.9
May	J.L.M.N.Y.Z.	18,000	87	5.8
June	U.J.L.N.Y.	9,000	33	4.4

miles of river fell from 929 in August 1951, to 17 in June 1952 which represents a fall from a density of 25 flies per mile to one of 0.5 flies per mile and eradication appears to be in sight. Once again expenses have been low, the DDT preparations used only costing £17 per mile.

The cost of labourers necessary for spraying over the 13 months was £120 and even if another £500 had to be spent on insecticide and £60 on labour until the end of 1952 the cost would still be under £40 per mile. It appears, therefore, reasonable policy to continue spraying the Ainamotua until eradication is achieved and then to continue along the main Nyando river until the whole area is covered.

As with hand-catching, so with DDT spraying, the areas under treatment must be well isolated and it is hoped that this can be achieved on the Nyando river basin. The river banks, from two miles downstream from Kibigori to the shore of the lake, a distance of 14 miles, are entirely free of *G. palpalis* owing to dense cultivation. This will isolate the whole area from permanent foci and spraying should therefore lead to elimination.

It is also worthy of note that in the area under review rain fell in every month from January 1951 to June 1952. In areas where a long dry season can be relied upon the efficacy of spraying should be increased. With low atmospheric humidity more *G. palpalis* may move along the river banks and the residual effect of the spraying would be longer.

Summary.

Of the various methods for the eradication of *G. palpalis* that have been tried in Kenya, hand-catching has the most limited application.

Total and discriminative bush-clearings of the river banks have been used on a considerable scale and progress on the Yala, Magori, and Nyando rivers is reviewed. The decrease in fly-populations caused by these clearings is gradual, but complete eradication may be achieved. Maintenance of the clearings is however difficult, the initial cost varied from £200 to £300 per mile and labour was often difficult to obtain in sufficient numbers.

The use of 5 per cent. DDT sprays on the fringing vegetation along 20 miles of the Mbogo river gave dramatic results and *G. palpalis* was eradicated at a cost of £42 per mile. The rate of application was 15 to 20 lbs. of DDT per acre and the residual effect lasted for at least 14 days during dry weather, but was rapidly lost during the rains.

Spraying along the Ainamotua gave equally promising results.

Areas under treatment, either by hand-catching, bush-clearing or spraying with DDT, must be properly isolated from other tsetse-infested areas if quick results are to be achieved.

Acknowledgements.

It is a pleasure to acknowledge the financial and other practical assistance given to us by the Colonial Insecticides Unit, Arusha, in the early stages of the spraying operations. Mr. Woodcock of the Unit remained at Kibigori camp to advise and assist us from February to May 1951. The bush-clearing operations prior to 1950 were carried out under the direction of Dr. Lewis.

Thanks are also due to our own Field Assistants, Messrs. Aitchison, von Bratt and Herbert, who were in charge of the operations at various times. This paper is published with the permission of the Director of Veterinary Services, Kenya.

References.

- GLASGOW, J. P. & DUFFY, B. J. (1947). Bull. ent. Res., **38**, pp. 465-477.
GLASGOW, J. P. & DUFFY, B. J. (1951). Bull. ent. Res., **42**, pp. 55-63.
MORRIS, K. R. S. (1946). Bull. ent. Res., **37**, pp. 201-250.
SYMES, C. B. & VANE, R. T. (1937). The eradication of *G. palpalis* from river areas by the "block" method.—61 pp. Nairobi, Govt. Printer.
WOODCOCK, K. E. (1949). Peripheral vegetation spraying—Ziribanje Island.—Bur. perm. interafr. Tsé-tsé Tryp., no. 79, 11 pp.
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A labourer using a knapsack sprayer applying DDT spray to the fringing vegetation in block T on the Mbogo river.

TESTS FOR INSECTICIDE-RESISTANCE IN LICE, MOSQUITOS AND HOUSE-FLIES.

By J. R. BUSVINE, D.Sc., and C. MARY HARRISON, Ph.D.

London School of Hygiene and Tropical Medicine.

The existence of insecticide-resistant strains of house-flies, body lice and culicine mosquitos is well established and there is some anxiety as to whether other insects may become similarly immune. The possibility of this development and the speed with which it would become evident, depends largely on the following factors:

- (i) The frequency of occurrence and the effectiveness of resistant genes in natural populations of the insect.
- (ii) The intensity of selection, i.e., the magnitude of the population exposed to insecticide and the proportion killed.
- (iii) The number of generations per year.

This paper deals primarily with problems associated with (i).

Most of the well-known cases of insecticide-resistance have been discovered in the field, after extensive use of a particular insecticide. Presumably, this would have had the effect of "developing" resistance by selective mortality of susceptible individuals over several generations.

Evidence on the speed with which resistance develops is not uniform. Thus, in most places where there has been extensive house-spraying, house-flies have been effectively controlled in the first season, with some failure in the second season and complete resistance in the third season. However, Hess (1952), referring to Wiesmann's early work on the subject, states that the flies at Arnas, Sweden were highly resistant *ab initio*. At the other extreme, it has been observed in India (Pal & Sharma, 1953) that resistance of flies (*Musca domestica nebulosa* F.) is slow to develop, despite considerable use of DDT. Again, in some parts of the U.S.A., DDT-resistant flies have been effectively controlled for two seasons with dieldrin, whereas in others a high resistance to the new compound developed within two months (Quarterman, 1950).

It is possible that these differences relate to the habits of the house-fly in different environments. If, for example, flies spent more time inside houses in some regions, they would then be more frequently exposed to insecticide. Alternatively, there may be variations in the initial degree of resistance (or frequency of resistant genes) in different areas of a species' distribution.

Evidence on the development of DDT-resistance in the human body louse is somewhat vague. It is not clear whether the failure of DDT to control lice in Korea (Hurlbut & others, 1952) followed wide and effective use of this insecticide, or whether the lice there were naturally more resistant than European strains. The DDT-resistant colony obtained from Egypt, on the other hand, may well have been started with lice from an area where DDT had been used intensively (Busvine, 1953).

The sections that follow present data relevant to the problem discussed above. Colonies of various insects from different sources (with or without previous histories of residual spraying) were tested for resistance to insecticides. Part I was done in 1948-49, by both of us, at the London School of Hygiene and Tropical Medicine. Parts II and III were carried out by one of us (J.R.B.) while on a visit to Nigeria in 1952.

I. Experiments with *Pediculus humanus corporis* Deg.

Between 1945 and 1949, a series of tests was conducted in this Department, on the louse-killing properties of artificial silk fabrics incorporating a standard quantity of DDT. The fabrics were made up as vests, which were worn by men engaged on a variety of activities, and washed weekly. The garments were tested after various periods of wear, ranging to 42 weeks.

At one point during this period (August 1947) the laboratory colony used in these tests was allowed to die out. Another colony was obtained from a naturally infested man in London in December 1947. The results of tests with this stock were inconsistent with those of the original colony; the new lice appeared to be more resistant. It was thought that this might have been due to improvements in the testing technique; but, alternatively, it was suggested that louse strains from different sources may vary in resistance to DDT. To investigate this possibility, new strains of lice were obtained from as many sources as possible, reared in the Department, and subjected to a standard test with the DDT-treated fabrics.

The louse colonies.

Body lice or their eggs, from about a dozen sources, were obtained from time to time, but only six of the strains survived and proliferated in the laboratory. The rearing methods, on the human leg, were as described by Buxton (1947). The following is a list of the colonies used in the tests:

Designation	Date obtained	Locality	Remarks
B	Dec. 1947	London	From infested man
C	Nov. 1948	"	" " "
D	Nov. 1948	Liverpool	" " "
G	Jan. 1949	Hamburg	Laboratory culture
I	Feb. 1949	Basle	" "
J	May 1949	Tunis	" "

Experimental method.

Six of the DDT-impregnated vests, worn for different periods, were taken as standards. They showed graded differences in insecticidal power. These standard fabrics were stored in a cupboard in darkness throughout the period of the investigation. From the known persistence of DDT at room temperatures in England, there was little likelihood of a decline in effectiveness during storage; and, indeed a survey of all tests showed no progressive decline in the efficiency of the standard fabrics.

For each test, two pieces of fabric, about one inch square, were cut out and the edges sealed with an alcoholic solution of shellac. The clippings were placed in a metal louse-feeding box, together with some adult lice and the tin was worn on the leg for four to six days. The lice were inspected for mortality daily and a final decisive count was made at the end of four days.

In the early part of this investigation several factors, which influenced the results of tests, were discovered and eliminated whenever possible. Thus, it was found that the resistance of adult lice declined with age; therefore, only three to four-day-old adults were used. Again, males were seen to be more susceptible than females; so equal numbers of the sexes were used in all subsequent tests.

Certain variable influences were very hard to control, because of difficulties inherent in rearing lice on human hosts. Several volunteers were available at different times, but it was not possible to use the same hosts throughout. Sometimes the number of lice available for tests was rather low; but the tests were always made on the complete range of fabrics, using a few lice per tin. The whole experiment was then repeated with a subsequent generation of the same stock, until results of about 30 lice per test fabric were collected.

Results.

The percentage kills of different strains of lice produced by the DDT-proofed fabrics are shown in Table I. In a statistical analysis, kindly performed by Dr. Armitage, these percentages were converted to angular transforms, to avoid

TABLE I.

Results of tests in which adult lice of different strains were exposed to two types of DDT-impregnated fabric, worn for different periods.

Louse strain	Percentage kill (numbers of lice used in brackets)						
	Fabric "X" worn for :			Fabric "Y" worn for :			Control fabric
	14 weeks	26 weeks	39 weeks	12 weeks	24 weeks	42 weeks	
B	71 (38)	27 (30)	32 (37)	27 (37)	28 (39)	20 (40)	12 (43)
C	95 (18)	40 (20)	13 (16)	36 (14)	29 (17)	13 (15)	0 (15)
D	65 (43)	14 (37)	18 (44)	34 (38)	21 (33)	6 (31)	0 (26)
G	87 (39)	17 (37)	6 (28)	45 (38)	44 (29)	0 (36)	7 (43)
I	64 (25)	31 (26)	4 (25)	68 (25)	67 (27)	23 (26)	5 (22)
J (total)	38 (96)	5 (97)	7 (106)	8 (95)	16 (91)	2 (96)	7 (87)
J i	47 (40)	12 (40)	14 (43)	16 (38)	30 (40)	5 (40)	15 (40)
J ii	53 (30)	0 (30)	13 (30)	3 (30)	7 (30)	0 (30)	0 (30)
J iii	8 (26)	0 (27)	0 (33)	4 (27)	5 (21)	0 (26)	0 (17)

the distortion of the variance at extreme values of the percentage range. An analysis of variance on these transformed variates showed highly significant differences ($P < .001$) between results with the different fabrics and between the different louse strains. The following list gives the mean transformed percentages of the louse strains, together with the standard errors.

Strain	Mean transformed percentage ± Standard Error	Corresponding percentages
B	35.3 ± 3.4	33
C	37.9 ± 5.1	38
D	29.8 ± 3.4	25
G	32.4 ± 3.4	29
I	39.6 ± 4.1	41
J	19.3 ± 2.1	11

It is now apparent that the only strain giving significantly different results from the others is "J", which appears to be abnormally resistant. If Table I is inspected, it will be seen that results of tests with three generations of "J" are given, each using considerable numbers. In the analysis described above, the data for the three generations were pooled. If, however, the results of the three generations are analysed separately, it is found that there are significant differences between them ($P < .01$). *between*

The statistical analysis confirms the impression gained by a careful inspection of the results. Of the six strains submitted to standard exposure on DDT-treated fabric, five showed no indication whatever of abnormal resistance. Mortalities of the sixth strain were significantly lower than the others, when the results of tests on three generations of this strain were combined. The colony concerned was derived from Tunis, where it had been cultured in Dr. Sparrow's laboratory for about twelve years. The validity of the high resistance of this strain is somewhat reduced by the fact that the results varied significantly between tests carried out on different generations.

II. Experiments with *Aedes aegypti* (L.).

Two matters were investigated: the variation in resistance of mosquito colonies obtained from widely different sources and the possibility of resistance developing in a locality where regular house-spraying with BHC had been practised for over two years.

1. Resistance of strains of *Aedes* from various sources.

The mosquito colonies.

During 1952, four colonies of *Aedes aegypti* from different sources were being maintained at the Virus Research Institute, Lagos, and these were kindly made available by the Acting Director, Dr. Macnamara. The colonies were started from eggs sent to Lagos as follows:

From Karachi, received 26 Nov. 1951
 „ Poona, „ 21 Jan. 1952
 „ Delhi, „ 26 Sept. 1951
 Local Nigerian colony, started 1 Dec. 1951.

The four colonies were reared in large cages, kept in screened compartments of an insectary, with mosquito gauze walls and a good insulating roof. Three mornings a week, a tied rabbit was put into each cage for an hour or two to provide blood meals.

It may be of interest here, to record some biological data for the four strains. The laboratory staff of the Virus Research Institute had been noting the dates on which egg papers were soaked and also the dates on which the first pupae and first adults were observed. Details of six generations of the Delhi strain, eight of the Nigerian and Poona and nine of the Karachi colony were available. From these records the following means and standard errors were calculated:

Strain				Days		Egg to Adult	
				Egg to Pupa			
Karachi	5.68	± 0.92	7.33	± 1.71
Poona	5.63	± 1.13	7.25	± 0.92
Delhi	5.60	± 0.90	7.00	± 1.00
Nigeria	6.13	± 0.96	7.88	± 0.85

Clearly no significant differences are apparent.

Experimental methods.

Female mosquitos were taken for tests two to four hours after feeding. Batches were exposed for one hour in cylinders made of filter paper impregnated with oil solutions of insecticide. The technique was almost identical with that of Busvine and Nash (1953) except that at Lagos the mosquitos were used shortly after a blood meal.

TABLE II.

Results of tests in which *A. aegypti* from different sources were exposed to residues of DDT oil solutions.

DDT (%)	Percentage kill (number used in brackets)					
	Series (i)				Series (ii)	
	Karachi	Poona	Delhi	Nigeria	Karachi	Nigeria
4	100 (26)	96 (46)	100 (58)	90 (59)	98 (88)	92 (84)
2	88 (129)	75 (52)	78 (41)	76 (72)	77 (62)	67 (52)
1	50 (82)	45 (44)	42 (33)	43 (41)	43 (44)	46 (54)
0.5	24 (37)	29 (28)	24 (29)	26 (23)	18 (62)	25 (60)

Results.

The results of the tests are summarised in Table II and shown as log. concentration/probit regression lines in fig. 1 (a). The percentages given in the Table are pooled figures for 4 to 12 batches of about a dozen individuals.

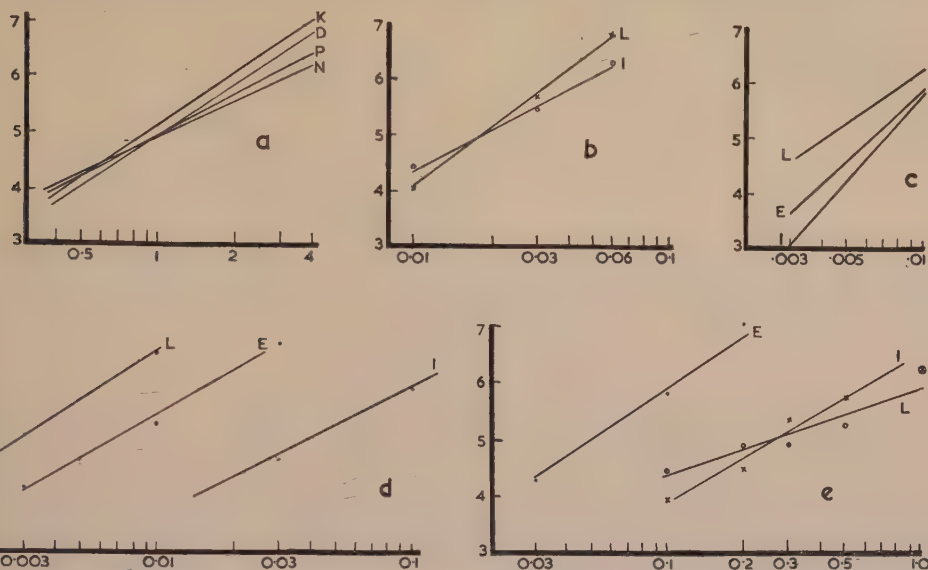


Fig. 1.—Log-concentration/probit regression lines indicating the relative resistance of strains of mosquitos and house-flies. In all cases, ordinates are probits and abscissae give concentrations of insecticides.

- (a) Resistance of four strains of *Aedes aegypti* to DDT. K = Karachi; D = Delhi; P = Poona and N = Nigeria.
 (b) Resistance to γ BHC of *Aedes aegypti* from Ilaro (I) and from Lagos (L).
 (c) Resistance of house-flies to dieldrin. (L) Lagos strain, (E) *M. domestica* from England, (I) Ilaro strain.
 (d) Resistance of house-flies to γ BHC. (Key as above.)
 (e) Resistance of house-flies to DDT. (Key as above.)

The first lot of tests on the four strains (series (i)) indicated considerable similarity in the resistance to DDT. The greatest difference seemed to be between the Nigerian and Karachi cultures. Further experiments were planned with these two strains, with new cultures more carefully standardised. The series (i) results were obtained with females of unknown ages, collected from the large permanent culture cages. New cultures of the Nigerian and Karachi colonies were started separately and adults from these were used not more than a week after emergence (series (ii)).

Statistical comments.

An analysis of variance on the probits of the percentage kills in each batch was kindly done by Dr. P. Armitage, who provided the following statistics:

	D.F.	Sums of Squares	Mean Square	Variance Ratio
(1) Regression Between slopes:	1	367.9778	367.9778	186
(2) Between series	1	0.4115	0.4115	< 1
(3) Within series	4	9.6995	2.4249	1.23
(4) About regression	12	9.9855	0.8321	< 1
(5) Within doses	93	183.9270	1.9777	1

The mean square for (5) is the heterogeneity factor and the fact that it is significantly > 1 shows that there is real variation between the replicate batches. On the other hand, the variation about the regression lines of the pooled batches for different doses (4) was unexpectedly lower than (5). It is difficult to know which term to use as the error variance for comparing regression lines, but it is safer to use (5), realising that, if anything, the error has been overestimated.

The following list of median lethal concentrations for the different strains shows no evidence of differences between them:

				Median lethal concentrations with 95% fiducial limits	
				Series (i)	Series (ii)
Karachi	0.87 (.69-1.07)	1.03 (.78-1.24)
Poona	1.03 (.77-1.26)	
Delhi	0.98 (.71-1.29)	
Nigeria	1.14 (.79-1.33)	1.11 (.86-1.41)

Series (i) and (ii) have been combined for the estimates of slopes of the regression lines, since the data were statistically consistent.

				Slope \pm Standard Error (Expressed in probits per unit log.-dose)	
Karachi	3.14 \pm	0.32
Poona	2.45 \pm	0.57
Delhi	3.14 \pm	0.56
Nigeria	2.20 \pm	0.33

The difference between Karachi and Nigeria is significant, especially as a somewhat high estimate of the error has been accepted.

Conclusions.

The experiments have shown that the four strains of *Aedes aegypti* show negligible differences in their mean resistance to DDT. There are, however, differences in the slopes of the regression lines, which reach significance in the extreme cases. It would be tempting to ascribe the flatter slope of the Nigerian strain to the presence of a small proportion of resistant individuals, which would give recoveries at high dosages. But this would involve a departure from a linear probit/log. dose regression line and there is no evidence for this. It seems, then, that the differences in slope merely express greater scatter of resistance in some populations than in others.

2. Tests for possible "acquired" resistance.

About 50 miles from Lagos is a small town called Ilaro, which has been the site of an anti-malaria residual spraying campaign conducted by the Nigerian Malaria Service. The town comprises some seven square miles of two roughly oval areas. There are about 11,000 inhabitants in some 3,000-4,000 dwellings. For two-and-a-half years prior to these experiments, every house in the town had been sprayed, at three-monthly intervals, with an aqueous BHC suspension to leave a deposit of 10-15 mg. γ BHC per square foot.

A. aegypti mosquitos were formerly very common, breeding in small pools of water, pots, gourds, etc. in the native compounds. Since the beginning of the spraying programme this mosquito has become comparatively rare, but larvae are still obtainable from breeding sites in the town.

It seemed worth investigating the effects of this house-spraying campaign on the resistance to BHC of the local population of *A. aegypti*. Since this is a very domestic mosquito, it is likely that a high proportion of the population would come into contact with insecticide in the houses and the resulting mortality should have a selective effect.

Colonies used and breeding methods.

A culture of *A. aegypti* was started at the Malaria Institute laboratory near Lagos with a score of larvae collected in Ilaro. They were reared to adults, fed on mice and about 200 eggs were collected on damp filter paper. These were dried for two days and then soaked in a breeding bowl at the same time as an egg batch from an old laboratory strain of *A. aegypti*, originally from Lagos. The two cultures were raised side by side and became adult at the same time. The adults were allowed to feed on mice when a few days old and were then subjected to insecticide tests. A second batch of about 200 eggs was obtained from the Ilaro colony and reared beside another of the Lagos strain. These were similarly used for tests when they became adult.

Experimental method and results.

The methods of Busvine and Nash (1953) were used as in the previous investigation with DDT. Three dosage levels were chosen and the percentage kills of mosquitos exposed to them are given in Table III. Probit/log. concentration regression lines are shown in fig. 1 (b).

TABLE III.

Results of tests in which *A. aegypti* from a sprayed and an unsprayed area were exposed to residues of γ BHC oil solutions.

Concn. (%) γ BHC	Proportions of mosquitos killed					
	Ilaro <i>Aedes</i>			Lagos <i>Aedes</i>		
	Test (i)	Test (ii)	Mean %	Test (i)	Test (ii)	Mean %
·06	14/14	19/20	97	9/10	22/24	91
·03	16/18	28/40	76	18/29	39/53	70
·01	2/14	3/12	19	6/19	6/20	31

A statistical analysis of the results obtained with the individual batches used in the tests showed no significant heterogeneity ($\chi^2 = 24.8$ with 22 d.f.) nor significant departure from linear regression. When the two series of tests were analysed separately to give four regression lines, there were no significant differences in median lethal concentrations and the variation in slopes did not quite reach significance at the 5 per cent. level.

Conclusions.

The sample of *A. aegypti* from the sprayed town of Ilaro showed no indication of increased resistance to BHC.

III. Experiments with *Musca domestica*, etc.

Colonies used in the tests.

The following cultures of flies were set up and maintained at the Malaria Service Laboratories near Lagos:

(i) *Musca domestica vicina* Macq. Collected from a market at Yaba, near Lagos.

(ii) *Musca domestica vicina*. Collected from Ilaro where, as stated earlier, a γ BHC house-spraying campaign had been in progress for two-and-a-half years.

(iii) *Musca domestica domestica* L. A normally susceptible strain was started with pupae flown out from our laboratory in England.

(iv) *Musca sorbens* Wied. Collected from the market at Yaba.

It may be of interest to record that, of the flies collected at Yaba market, those captured in the full daylight were mainly *M. sorbens*, while those caught in the shade of the market stands were mainly *M.d. vicina*.

Rearing methods.

It was found impossible to follow the standard laboratory methods for breeding houseflies. For one reason, the locally caught *M.d. vicina* refused to lay eggs on cotton wool pads soaked in milk and water (only diluted tinned milk was available, but the *M.d. domestica* strain laid readily on this). Secondly, a mixture of cereal and dried grass meal, suitable for preparing larval food, could not be obtained locally. Powdered cassava was tried, alone or mixed with groundnut flour or dried coconut meal, but it produced only a few stunted flies. For these reasons, monkey faeces were used throughout the investigation for eliciting oviposition and as larval food. Good sized flies of all strains were obtained within about 10 days from hatching at laboratory temperatures (80–84°F.). Lumps of sugar and cotton wool pads soaked in milk and water were provided as food for the adults, which survived very satisfactorily in all strains.

Experimental results.

The technique used to measure resistance in the various strains was exactly the same as in previous work in England (Busvine, 1951, 1953). Batches of flies were dosed individually with drops of oil solutions of insecticides applied

TABLE IV.

Results of tests with insecticides on various strains of house-flies.

Each percentage calculated from about 30 flies of the Ilaro *vicina* and *M. sorbens* strains and 50 flies of the other two strains.

Insecticide	Concentration %	Mean percentage kill			
		Ilaro <i>M. vicina</i>	Yaba <i>M. vicina</i>	English <i>M. domestica</i>	Yaba <i>M. sorbens</i>
Dieldrin	0.03	100	100	100	
	0.01	78	89	80	
	0.003	3	35	9	
M.L.C.		0.007 c.	0.004 c.	0.006 c.	
<i>gamma</i> BHC	0.1	81			
	0.03	35	100	97	
	0.01	18	95	59	
	0.003		45	20	100
	0.001		2		43
M.L.C.		0.04	0.0035	0.007	0.001 c.
DDT	1.0	89	89		
	0.5	73	63	100	
	0.3	66	47	100	100
	0.2	32	40	100	—
	0.1	15	30	80	100
	0.03			26	54
M.L.C.		0.27	0.27	0.050	0.03

dorsally with a micro-syringe. The results of the experiments are given in Table IV. Average mortalities of males and females were calculated and the means for each point are shown as probits plotted against concentrations on a logarithmic scale in fig. 1 (c), (d), (e). The results show that:—

(i) The *M.d. domestica* strain gave results consistent with similar tests on this strain in England.

(ii) Compared with this strain, the *M.d. vicina* from Yaba were rather more susceptible to γ BHC and dieldrin, and the *M. sorbens* flies were considerably more susceptible to DDT and γ BHC. All these differences could be explained merely by the average sizes of the flies; for the *M.d. vicina* flies were slightly smaller than the *M.d. domestica* and the *M. sorbens* were considerably smaller.

(iii) Both the Ilaro and Yaba colonies of *M.d. vicina* were decidedly resistant ($\times 5.4$) to DDT compared with the *M.d. domestica* from England. This is most interesting, because there appears to have been no systematic house-spraying by the authorities in Lagos and it is unlikely that much DDT has been used by the inhabitants. No DDT was used in the house-spraying at Ilaro.

(iv) The Ilaro *M.d. vicina* were clearly resistant to γ BHC compared to the Yaba *M.d. vicina* ($\times 11.4$), which is not surprising in view of the γ BHC-spraying programme at Ilaro. The Ilaro *M.d. vicina* appear to be about twice as resistant to dieldrin as the Yaba flies. The data are rather meagre, but it is clear that this enhanced resistance is much less than that towards γ BHC.

General Summary and Conclusions.

Between December 1947 and May 1949, six colonies of body lice from various sources were cultured in the laboratory. Three of the strains originated from natural infestations in England and the others came from laboratory cultures in Hamburg, Basle and Tunis. After being bred for at least one generation in the laboratory, these lice were subjected to a standard test for resistance to DDT. Only one strain showed any evidence of abnormal resistance; this was the strain from Tunis where it had been laboratory-cultured for over 12 years.

Colonies of *Aedes aegypti* from Karachi, Poona and Delhi were being cultured at the Virus Research Institute, Lagos, together with a local Nigerian strain. Extensive tests showed no difference in average susceptibility of the four strains to DDT, though there was some evidence of differences in the spread of resistance through the various populations.

A colony of *A. aegypti* was started with larvae taken from Ilaro, a Nigerian town which had been regularly sprayed with BHC for $2\frac{1}{2}$ years. Comparative tests showed no difference in resistance to γ BHC between this colony and a laboratory culture at the Malaria Service Laboratory, Lagos.

Colonies of house-flies were initiated and maintained at Lagos. Tests with insecticides gave the following results:

(a) *M.d. vicina* from Ilaro were much more resistant to γ BHC than similar flies from Yaba, near Lagos, or *M.d. domestica* from England.

(b) The *M.d. vicina* from both Lagos and Ilaro were considerably more resistant to DDT than *M.d. domestica* from England. This is surprising, as no DDT-spraying has been done at Ilaro and, so far as can be ascertained, virtually none in Lagos.

(c) The *M.d. vicina* flies from Lagos were considerably more susceptible to γ BHC and dieldrin than *M.d. domestica* from England. *M. sorbens* was much more susceptible to γ BHC and to DDT. These differences could be explained by the smaller average sizes of the two susceptible species.

Acknowledgements.

We are grateful to those people who sent samples of lice or their eggs from various sources: also to Dr. P. Armitage who was of great assistance with the statistical analysis.

The senior author is particularly grateful to Dr. L. J. Bruce-Chwatt for offering accommodation and other facilities during his stay in Lagos.

References.

- BUSVINE, J. R. (1951). *Nature*, Lond., **168**, pp. 193-195.
- BUSVINE, J. R. (1953). *Ibid.*, **171**, pp. 118-119.
- BUSVINE, J. R. & NASH, R. (1953). *Bull. ent. Res.*, **44**, pp. 371-376.
- BUXTON, P. A. (1947). *The Louse*. 2nd edn.—164 pp. Arnold, London.
- HESS, A. D. (1952). *Amer. J. trop. Med. Hyg.*, **1**, pp. 371-388.
- HURLBUT, H. S., ALTMAN, R. M. & NIBLEY jr. C. (1952). *Science*, **115**, pp. 11-12.
- PAL, RAJINDAR & SHARMA, M. I. D. (1953). *Trans. IX int. Congr. Ent.* Amsterdam, 1951, **2**, pp. 347-350.
- QUARTERMAN, K. D. (1950). *CDC Bull.*, **9**, no. 11, pp. 3-7. (Quoted by HESS.)
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AN ECOLOGICAL STUDY OF THE INSECTS AND MITES IN THE NESTS OF CERTAIN BIRDS IN BRITAIN.

By G. E. WOODROFFE.

*Department of Scientific and Industrial Research,
Pest Infestation Laboratory, Slough, Bucks.*

E.M.

(PLATES XIV, XV and XVI.)

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The work upon which this paper is based was carried out in England during the autumn and winter of 1950, and the early spring of 1951. Previously, during the first three months of 1950, some exploratory work was done, and the results have already been published (Woodroffe & Southgate, 1951a). In addition, two short notes have been published which deal with specific points (Woodroffe, 1950; Woodroffe & Southgate, 1951b). One of the most important items in the literature on this subject is the paper by Linsley (1944) in which he gathers together many scattered records, contributes a number of his own, and summarises the position at that date. Most of the available records of insects occurring in birds' nests can be traced through the references given by that author and by Hinton (1945). However, one paper seems to have escaped the notice of both these workers, and the writer is indebted to Mr. G. B. Thompson for drawing his attention to it. This is the work of Nordberg (1936), and it is the most important contribution that has so far been made towards our knowledge of nest fauna. Nordberg's paper will be summarised and his results examined in the last section of this paper.

A recent publication by Weidner (1952) deals with the insect ecology of the city of Hamburg. This contains many references to nest insects and includes a useful bibliography. Weidner, also, has overlooked Nordberg's work.

The general background to the present work has been given in the earlier paper, and so will be mentioned only briefly here. The survey was conducted with the following three points as its chief aims:—

1. The collection of sufficient information on nest fauna to enable an assessment to be made of the importance of birds' nests as reservoirs of domestic and storage pests in Britain.

2. The study of the habits and behaviour of pest species in the nest habitat in order to supplement or confirm information gained from laboratory life-history studies.

3. The study of the nest as a microhabitat.

The facts presented in this paper add to existing knowledge on all three points.

The most important conclusion reached during the preliminary work was that the humidity conditions within a nest are of primary importance in determining the composition of the scavenging fauna. Nests may be classified as "wet" or "dry" according to whether they are exposed to, or protected from, rain or drainage water. The effect of humidity conditions upon the composition of the insect fauna will be discussed in detail later. It is only necessary here to explain that, having established the difference between the two types of nest, this survey covered only "dry" nests and, unless a statement to the contrary is made, it is this type of nest which is referred to throughout the paper.

METHODS.

The method of examining material has remained substantially the same as that described previously (Woodroffe & Southgate, 1951a). It consisted of sieving the disintegrated nest material and warming the various fractions on a tray over a hot-plate. The use of such methods as the Berlese funnel were found to be impracticable for several reasons. The quantity of material to be examined was often very large and the presence of material varying from large twigs to extremely fine dust necessitated a considerable amount of preliminary separation; many insects were present in an inactive stage, and some, such as the case-bearing Tineid larvae, experienced considerable difficulty in moving rapidly in a definite direction through the type of material that contained them; also, it was found that some insects—*e.g.*, Dermestid larvae—could be driven out only by heat treatment which would be rapidly lethal to other insects, such as lepidopterous larvae. In any case, it was often necessary to rear the adults in order to identify some of the species with certainty, and this precluded the use of any automatic method of separation which involved killing the insects.

In the absence of a suitable method of automatic collection of high efficiency, it was not possible to obtain precise quantitative information concerning the degree of infestation of each nest. Such figures would, in any case, be misleading, because even if nests are examined at the same time, the insect populations need not necessarily be in corresponding stages of development. Very large numbers of small larvae of a particular species could represent the same degree of infestation as much smaller numbers of full-grown larvae of that species if mortality in the early larval stages was normally high. Consequently, it was found more satisfactory to use a standard method of examination of nest material and to form a general opinion of the abundance of each species during the process of examination and collection. As the examination of each nest was completed, these estimates were recorded on a standard form by means of arbitrary symbols. Details of position and composition, and also subsequent identifications, were recorded on the same form. In one instance, a complete count was made of all the insects from a house-sparrows' nest in order to obtain some idea of the numerical value of the arbitrary estimates. Table I gives approximate values of

TABLE I.
Approximate numerical values of arbitrary estimates of abundance of several species of insects in a single nest.

Arbitrary estimate of abundance	Abbreviation used in records	Approximate numbers per nest									
		<i>Hofmannophila pseudospretella</i>	<i>Tinea columbariella</i>	<i>Anthrenus verbasci</i>	<i>Attagenus pellio</i>	<i>Tenebrio molitor</i>	<i>Pinus tectus</i>	Other <i>Pinids</i>	<i>Lepisma saccharina</i>	<i>Scenopinus fenestralis</i>	<i>Lyctocoris campestris</i>
Very large numbers	VLN	500-1000	1000-3000	100-200	100-500	200-500	200-500	50-150	100-500	50-100	100-200
Large numbers	LN	100-500	500-1000	50-100	50-100	100-200	100-200	30-50	50-100	30-50	50-100
Moderate numbers	MN	50-100	100-500	20-50	20-50	50-100	50-100	20-30	20-50	20-30	20-50
Small numbers	SN	20-50	50-100	10-20	10-20	20-50	20-50	10-20	10-20	10-20	10-20
Very small numbers	VSN	<20	<50	<10	<10	<20	<20	<10	<10	<10	<10

these estimates for some of the more important insect species, all stages other than eggs being included. For some species (*Hofmannophila*, *Tenebrio*, *Ptinus tectus* Boield., *Lepisma*, *Lyctocoris*), the highest estimates were reached only in pigeons' nests, where it was often impossible to define exactly the limits of a single nest. The highest estimates for *Tinea columbariella* (Wocke) and *Anthrenus verbasci* (L.) usually occurred in house-sparrow nests.

FAUNAL LISTS.

Throughout the faunal lists, the species have been dealt with in one of three ways:—

1. Where a species is extremely abundant and widespread in nests, individual records would involve listing the localities of most of the hundreds of nests examined; consequently, only a general statement of the status of the species has been made, and only records of some special interest or significance have been detailed individually. The frequency of occurrence and abundance of most of such species are illustrated in fig. 1.

2. Certain groups of closely related species (e.g., the Cryptophagid and Lathridiid beetles) have little importance as nest inhabitants or warehouse pests; such groups have been dealt with as units and detailed records for individual species have not been given.

3. Otherwise, where an insect is uncommon, its habitat unusual, or its distribution likely to be of interest to workers in the group, details of bird species, position of nest, locality, date and abundance* have been given for each record.

THE INSECT FAUNA.

(a) Ectoparasites of Birds.

From the point of view of this survey, the bird parasites have little importance except as possible prey of predatory species. However, a number of records, particularly of the Cimicid bug, *Oeciacus hirundinis* (Jen.), and the Hippoboscid, *Stenopteryx hirundinis* (L.), have accumulated, and these may be of interest to those concerned with parasites. Detailed records of most parasitic species have been sent to Mr. G. B. Thompson for inclusion in his forthcoming publications. Consequently, only a simple list of species is presented here.

Hemiptera.

CIMICIDAE.—*Oeciacus hirundinis* (Jen.).

Diptera.

CALLIPHORIDAE.

Protocalliphora azurea (Fall.). This species was found to be very widespread in the nests of many species of birds. It appeared to thrive particularly in those of swallows. The puparia were frequently heavily parasitised by the Pteromalid, *Mormoniella vitripennis* (Walk.).

HIPPOBOSCIDAE.

Ornithomyia fringillina Curt., *Ornithomyia avicularia* (L.), *Stenopteryx hirundinis* (L.), and *Crataerina pallida* (Latr.). Two puparia of *C. pallida* were parasitised by *Dibrachys* sp. (Hym. PTEROMALIDAE). It has been possible to discover only very few other records of a successful attack by Hymenopterous parasites upon puparia of the Hippoboscidae, and none by *Dibrachys*.

* A key to the abbreviations used for the estimates of abundance is included in Table I.

Siphonaptera.

Ceratophyllus hirundinis (Curt.), *C. farreni* Roths., *C. columbae* Gerv., *C. gallinae* (Schr.), *C. fringillae* Walk. and *Dasypsyllus gallinulae* (Dale).

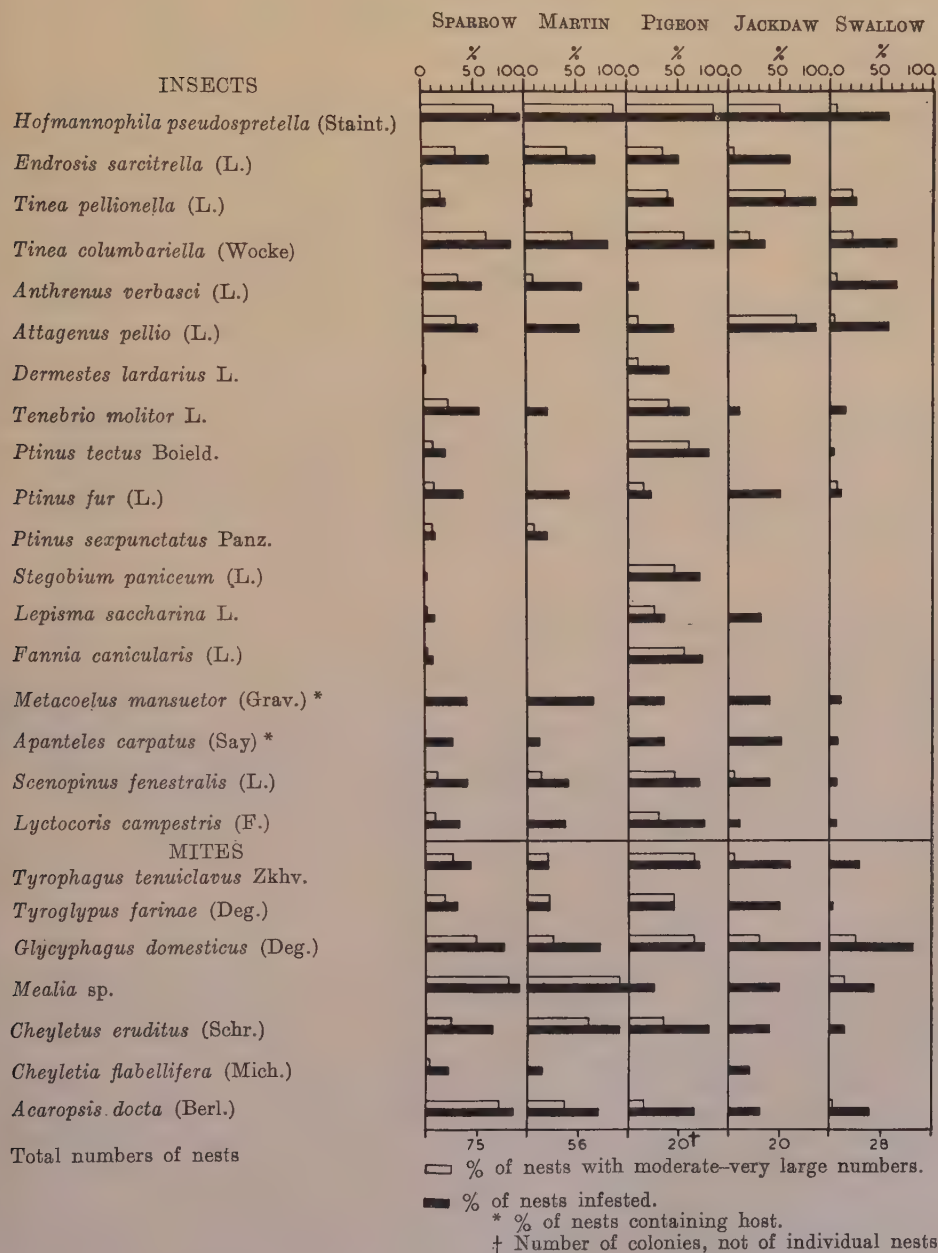


Fig. 1.—Frequency and abundance of some nidicoles in the nests of five bird species.

(b) Scavengers.**Thysanura.****LEPISMATIDAE.**

Lepisma saccharina L. The common silverfish occurred only occasionally in birds' nests in general, but sometimes very large numbers occurred in pigeon's nests. It is surprising to find this supposed starch feeder thriving in solid pigeon guano.

Lepidoptera.**PHYCITIDAE.**

Ephestia elutella (Hb.). A few larvae were occasionally found in sparrows' nests into which they had probably wandered from some other source. This is a major pest of grain, but there is no evidence that it can breed in nests in Britain.

Records. Sparrow, eaves of Parrot House, Regent's Park, London, 29 Sep. 50, VSN; sparrow, eaves of stables, farm at Wickham Bishops, Essex, 29 Nov. 50, VSN.

PYRALIDIDAE.

Pyralis farinalis (L.). *P. farinalis* is a minor pest of stored grain in Britain. It has been found only in pigeons' nests, but sometimes in some numbers.

Records. Pigeon (many nests), belfry, Chelmsford Police Station, Essex, 29 Nov. 50, MN; pigeon (many nests), bombed storage shed, Bristol, 18 Apr. 51, SN.

OECOPHORIDAE.

Hofmannophila pseudospretella (Staint.). The Brown House Moth is one of the commonest indoor moths in Britain. It has some importance as a general pest, attacking a wide variety of materials when the humidity is sufficiently high (Woodroffe, 1951), and may act as a grain pest or as a clothes moth. It is probably the commonest species found in nests, being absent only from some very dry swallows' nests, and is often present in very large numbers.

Endrosis sarcitrella (L.). This species, the White-shouldered House Moth, is very similar in importance to the last. It is often reported damaging stored beans and peas. It is generally less widespread and abundant in nests than *H. pseudospretella*, but may become dominant in nests such as those of titmice, which contain an abundance of green moss.

TINEIDAE.

Monopis rusticella (Clerck). This species is never an important pest, but is commonly found associated with stored products. It may be described as an intermediate species, occurring in both "wet" and "dry" nests, and was found principally in pigeons' nests.

Monopis weaverella (Scott). Ford (1949) summarises our knowledge of this species in five words—"In woods and on heaths." It was reared twice from pigeons' nests, both from the centres of large towns, and once from a jackdaws' nest in the country.

Records. Pigeon (many nests), turret, roof of hotel, Russell Sq., London, 25 Jan. 51, SN; pigeon (many nests), bombed storage shed, Bristol, 18 Apr. 51, VSN; jackdaw (several nests), hollow tree in meadow land, East Bergholt, Suffolk, 5 Oct. 50, VSN.

Monopis crocicapitella (Clemens). This species is sometimes associated with stored products of animal origin, and occasionally occurs in houses. Although seldom found in large numbers, it has, on one occasion, caused a major infestation (Woodroffe & Southgate, 1952). The larvae are case-bearers, and were found several times in some numbers in pigeons' nests.

Records. Pigeon (many nests), Railway Docks, Brentford, Middx., various situations, 15 Nov. 50, SN; pigeon (many nests), bombed storage shed, Bristol, 18 Apr. 51, MN.

Tineola bisselliella (Hummel). The Common Clothes Moth cannot be considered a regular nest-dwelling species in Britain, although its larvae have been found in nests in small numbers on several occasions. Its position is discussed later in this paper.

Records. Sparrow (2 nests), eaves of house, Upton Park, Slough, Bucks, 4 Aug. 50, SN; sparrow (2 nests), eaves of buildings, Pest Infestation Lab., Slough, Bucks, 10 Aug. 50, SN & VSN; sparrow, eaves of house, Datchet, Bucks, 23 Aug. 50, VSN; sparrow, crevice in wall of bombed house, Neal Street, Covent Garden, London, 23 Nov. 50, VSN.

Tinea fuscipunctella Haw. This is another species which is casually associated with stored products, though it has no economic importance. It was found only twice, once in a sparrows' nest and once in pigeon guano.

Records. Sparrow, eaves of house, Wallington, Surrey, 29 Aug. 50, VSN; pigeon (many nests), Railway Docks, Brentford, Middx., 15 Nov. 50, VSN.

Tinea pallescentella Staint. The Large Pale Clothes Moth is rather more important as a domestic nuisance than the previous species. It was found in considerable numbers in pigeons' nests at Brentford (MN) and Bristol (LN). (Records as for *M. crocicapitella*.)

Tinea pellionella (L.). The Case-bearing Clothes Moth is second only to *Tineola bisselliella* as a pest of woollen fabrics. It is abundant and widespread in nests, especially those of jackdaws and pigeons.

Tinea columbariella (Wocke). This species also has case-bearing larvae and in the past has probably been confused with *T. pellionella*. It has been found attacking clothing in company with the previous species (Woodroffe, 1950), but the extent to which it is a clothes moth is not yet known. It is very widespread in nests and is usually the dominant species in sparrows' nests. 7

Coleoptera.

DERMESTIDAE.

Dermestes lardarius L. The Bacon Beetle is a common minor pest of animal products. It occurs widely in pigeons' nests in London, but the record quoted is the only occasion when it was found elsewhere.

Record. Sparrow, eaves of house, Wallington, Surrey, 29 Aug. 50, VSN.

Attagenus pellio (L.). The Fur Beetle is a clothing pest of some importance. It occurs frequently in nests, and is sometimes very abundant, particularly in jackdaws' nests. It appears less dependent upon the presence of buildings for reaching high population densities than many of the other nest species (see record below). When a dry nest is abandoned by the birds and never used again, it becomes slowly converted into a mass of fine dust (mostly insect faecal pellets) in which the coarser fibres (e.g., straw) remain. Nests in this condition rarely contain much life, but when insects are present, *A. pellio* is usually dominant. It appears to be able to utilise material which has become unsuitable for most other species.

Record. Jackdaw (several nests), hollow tree in meadow land, East Bergholt, Suffolk, 5 Oct. 50, VLN.

Megatoma undata (L.). This Dermestid is usually found under the bark of trees feeding on the remains of dead insects. It has been known to damage hides. Larvae were found in a nest on one occasion.

Record. Robin, in shed, Pest Infestation Laboratory, Slough, Bucks, 28 Oct. 51, SN.

Anthrenus verbasci (L.). The Varied Carpet Beetle is a domestic clothing pest that seems to be increasing in importance in many areas. It is a common nest species, but is practically confined to those of sparrows, martins and swallows.

Anthrenus fuscus Oliv. This species is only a very minor pest; it seldom occurs in nests, and was found in them on only two occasions.

Anthrenus museorum (L.). The Museum Beetle is best known for its depredations in insect collections, although *A. verbasci* is probably as important in this respect. Larvae were only occasionally found in nests, usually in company with those of *A. verbasci*.

(The whole problem of the comparative distribution of these three species of *Anthrenus* is now under examination. By collecting adults from flower-heads in summer some evidence has already been obtained of differences in distribution which can be attributed to differences in type of locality (*e.g.*, suburban areas as opposed to rural areas). By collecting larvae from a variety of situations, in addition to birds' nests, some evidence of differences in larval habitat has been obtained. This work is still in progress and will be reported in a separate publication which will include detailed records of *Anthrenus* in nests.)

CUCUJIDAE.

Oryzaephilus surinamensis (L.). This major pest of grain was once found in numbers in sparrows' nests on an Essex farm. Only adults were present, and there was no evidence that breeding took place, or that the species overwintered successfully in this habitat. The insects had probably spread from some infested feeding-stuff, but, from the numbers present, it appeared that they had been attracted to the nests. Infested nests were found in a number of separate buildings on the farm.

Ahasverus advena (Waltl.). This species is a minor pest of a very wide variety of materials. It was found only once in nests.

Record. Pigeon (many nests), Railway Docks, Brentford, Middlesex, 15 Nov. 50, SN.

ANOBIIDAE.

Stegobium paniceum (L.). The Biscuit Beetle is a pest of some importance. It attacks a wide variety of both animal and vegetable products, and frequently causes damage to herbarium specimens. It was one of the dominant species in pigeons' nests in London, but was only once found in nests of other birds.

PTINIDAE.

Mezium affine Boie. This species is one of the rarer Ptinids, and is seldom found in any numbers on stored products. It occurred only twice in nests and on each occasion there was an obvious nearby source of the insects. It cannot be regarded as a typical nest species in Britain.

Records. Sparrow, ventilator, Parrot House, Regent's Park, London, 29 Sep. 50, VSN; pigeon (many nests), Railway Docks, Brentford, Middlesex, 15 Nov. 50, VSN.

Trigonogenius globulus Sol. This minor pest of grain and cereal products was confined to pigeons' nests in London and Bristol, where it occurred in large numbers.

Records. Pigeon, under 5th floor balcony, Strand, London, 31 Oct. 50, SN; pigeon (many nests), behind masonry, front of Charing Cross Station, London, 31 Oct. 50, VLN; pigeon (many nests), old sack hoist, clothing store, Peckham Rye, London, 31 Oct. 50, VLN; pigeon (four nests), on beams in transit sheds, Portishead Docks, Somerset, 19 Apr. 51, SN; pigeon (many nests), bombed storage shed, Bristol, 18 Apr. 51, VLN.

Niptus hololeucus (Fald.). The Golden Spider Beetle is a common domestic pest, occurring in private houses in small numbers, and occasionally giving rise to major warehouse infestations. It occurred sporadically in nests in small numbers, and the adults show a peculiar preference for rubbish such as mortar rubble, which is often closely associated with some nests, rather than for the nests themselves. Large numbers of adults (mostly dead) were found in such circumstances near jackdaws' nests high up on the roof of Canterbury Cathedral.

Pseudeurostus hilleri (Reitt.). This is an introduced but established species which occurs on stored products, principally in the north of the country. It was found in a nest once.

Record. Pigeon, on beams outside warehouse, Birkenhead, Cheshire 31 Dec. 51, VSN.

Ptinus fur (L.). This species, the White-marked Spider Beetle, is more widely distributed in nests than any other species of Ptinid. The extent to which this may be attributed to its ability to fly is discussed in a later section. It occurs widely on stored products but is of minor importance only. The record quoted illustrates its presence in areas far removed from buildings.

Record. Shelduck, on ground under hawthorn bush (*Crataegus*) in meadow, East Bergholt, Suffolk, 5 Oct. 50. VSN.

Ptinus pusillus Sturm. This occurs in warehouses in small numbers, usually in company with *P. fur*, and its distribution in nests is similar. In one remarkable instance, a pigeons' nest which was taken from a building in the centre of Maidstone, Kent, and consisted of no more than a handful of twigs and a few lumps of guano, contained 130 adults of *P. pusillus*. No other species of Ptinid was present.

Records. Sparrow (2 nests), eaves of house, Slough, Bucks, 14 Aug. 50, SN; swallow (2 nests), outhouse of farm, Wickham Bishops, Essex, 12 Sept. 50, VSN; swallow (2 nests), farm, Stanwellmoor, Middx., 11 Oct. 50, VSN; sparrow, byre of farm, Wickham Bishops, Essex, 29 Apr. 50, VSN; house-martin (2 nests), eaves of house, Nursling, nr. Southampton, 19 Jan. 51, VSN; pigeon (4 nests), on piles of derelict wharf, Portishead Docks, Somerset, 19 Apr. 51, SN; pigeon (many nests), bombed storage shed, Bristol, 18 Apr. 51, SN; sparrow, eaves of house, Canterbury, Kent, 27 Nov. 51, VSN; pigeon, eaves of Coach Museum, Maidstone, Kent, 27 Nov. 51, VLN; jackdaw (many nests), roof of nave, Canterbury Cathedral, Kent, 27 Nov. 51, MN.

Ptinus subpilosus Sturm. This is a rare species, and its association with stored products is doubtful. Single specimens were found in nests on two occasions.

Records. Sparrow, eaves of house, Wallington, Surrey, 29 Aug. 50, VSN; shelduck, on ground under hawthorn bush (*Crataegus*) in meadow, East Bergholt, Suffolk, 5 Oct. 50, VSN.

Ptinus tectus Boie. The Australian Spider Beetle is the most important Ptinid pest in Britain. It is one of the dominant species in pigeons' nests and

occurs also, but less commonly, in sparrows' nests. Its status will be discussed more fully later in this paper.

Ptinus sexpunctatus Panz. This is a rare insect, usually associated with bees (Linsley, 1944). It is the only Ptinid which flies freely in this country. Occasional specimens have been found in nests, and in one locality (Bedford) it was abundant (see also pp. 760-761).

Records. Sparrow, eaves of house, Addiscombe, Surrey, 29 Aug. 50, VSN; sparrow, eaves of house, Bedford, 5 Sept. 50, LN; sparrow (4 nests), eaves of house, Bedford, 5 Sept. 50, MN; house-martin (4 nests), eaves of house, Bedford, 5 Sept. 50, LN; sparrow, eaves of Flatford Mill, Suffolk, 6 Oct. 50, VSN; house-martin (2 nests), eaves of house, Nursling, nr. Southampton, 19 Jan. 51, VSN.

TENEBRIONIDAE.

Tenebrio molitor (L.). The Yellow Meal Worm is rarely more than a nuisance on cereals. It is an important nest species and is widely distributed; it reaches its peak abundance in pigeons' nests.

Tenebrio obscurus Fab. The Dark Meal Worm occurred once in a sparrows' nest on an Essex farm. It has a similar status as a pest to *T. molitor* but is much less common.

Trox scaber (L.). This species is best known as an inhabitant of owls' nests, and it has been found associated with jackdaws. It sometimes occurs in slaughterhouse waste.

The following species of Coleoptera belonging to several families have been found in nests, mostly in pigeons' nests. They are probably all mycetophagous and are widely distributed in haystacks and vegetable refuse of many kinds. They are all associated with stored products, where they feed on moulds in damp corners of warehouses, or on the products themselves where these have been allowed to become mouldy. They are of no economic importance and differ from typical dry-nest species in their wide distribution elsewhere in a variety of habitats. Consequently they are merely listed here, without individual records.

CRYPTOPHAGIDAE.

Henoticus californicus (Mann.), *Cryptophagus scanicus* (L.), *C. saginatus* Sturm., *C. subfumatus* Kraatz., *C. scutellatus* New., *C. distinguendus* Sturm. (including *umbratus* Erich.), *C. pallidus* Sturm., *C. acutangulus* Gyll., *C. cellaris* (Scop.) and *C. postpositus* Sahl.

LATHRIDIIDAE.

Lathridius bergrothi Reitt., *Enicmus minutus* (L.), *Cartodere filiformis* (Gyll.), *C. ruficollis* (Marsh.), *Corticaria pubescens* (Gyll.), *C. fulva* Com., *C. crenicollis* Mann., *Corticaria gibbosa* (Herbst) and *C. fuscula* (Gyll.).

MYCETOPHAGIDAE.

Mycetophagus quadripustulatus (L.).

COLYDIIDAE.

Murmidius ovalis (Beck).

ENDOMYCHIDAE.

Mycetaea hirta (Marsh).

Diptera.

In general, Diptera are not typical members of the dry-nest fauna. They require moist conditions and are more frequent in the wet type of nest. Those that were found were mostly confined to pigeons' nests and especially to those in which some excess moisture occurred. *Fannia canicularis* was apparently the only species which could tolerate the humidity conditions of sparrows' nests, and it was found only occasionally and in small numbers in such situations. The infrequency with which most of the Diptera have been recorded is also due to the fact that larvae are difficult to rear, and usually cannot themselves be identified beyond the family or genus.

ANISOPODIDAE.

Anisopus fenestralis (Scop.) was found once in a flycatchers' nest.

SCATOPSIDAE.

Scatopse notata (L.) was bred once in some numbers from a sparrows' nest from London.

HELOMYZIDAE.

Tephrochlamys tarsalis Zett. This carrion fly is one of the commonest Diptera found in wet nests, and occurred frequently and in moderate numbers in pigeons' nests.

CALLIPHORIDAE.

Sarcophaga barbata Thoms. This common flesh fly is frequently found indoors, but is of little economic importance. It is widespread and sometimes abundant in pigeons' nests.

Calliphora erythrocephala (Meig.). This is one of the "blowflies" and has considerable importance in slaughterhouses and wherever food is exposed to it. It is fairly widespread in pigeons' nests.

Pollenia rudis Fab. The Cluster Fly was recorded twice from house-martins' nests.

MUSCIDAE.

Musca domestica L. Conditions in dry nests are fortunately seldom suitable for larvae of the Common House-fly. It was found on only two occasions, in each case in a house-martins' nest. Only small numbers were present.

Fannia canicularis (L.). The Lesser House-fly is the commonest species of Diptera occurring in dry nests. It was found in sparrows' nests, but reached its peak abundance in pigeons' nests, of which it was one of the characteristic species. It has not the medical importance of the Common House-fly.

Helina uliginosa (Fall.) was found occasionally in pigeons' nests in London.

Anthomyia pluvialis (L.) was found once in a sparrows' nest in London.

(c) Predators and Parasites.**Hemiptera.****REDUVIIDAE.**

Empicoris culiciformis (Deg.). This bug occurs in warehouses as a predator of small insects and, probably, mites. It has seldom been reported as it is very difficult to see, and even when observed could be easily mistaken for a mosquito.

It is probably commoner than the records indicate. It sometimes occurred in sparrows' nests.

Records. Sparrow, eaves of building, Pest Infestation Laboratory, Slough, Bucks, 10 Aug. 50, VSN; sparrow, eaves of house, Datchet, Bucks, 23 Aug. 50, VSN; sparrow, eaves of old water tank, Regent's Park, London, 29 Sept. 50, VSN; sparrow, under sack hoist, Flatford Mill, Suffolk, 5 Oct. 50, VSN; sparrow, stables, farm, Witham, Essex, 29 Nov. 50, VSN.

Reduvius personatus (L.). This was an uncommon species in nests; it is now found as a general warehouse predator, though seldom in large numbers. It occurred in a derelict theatre at Guildford, Surrey, in association with pigeons. The record quoted below is of special interest as being one of the rare occasions on which *R. personatus* has been found breeding out of doors. The specimen found was a nymph, probably in the second instar.

Record. Jackdaw, hollow tree in meadow land, East Bergholt, Suffolk, 5 Oct. 50, VSN.

ANTHOCORIDAE.

Lyctocoris campestris (Fab.). This bug is the commonest predator found in nests and in warehouses. It is also widely distributed in a variety of other habitats, such as haystacks. In dry nests, it feeds principally upon House-Moth larvae.

Coleoptera.

HISTERIDAE.

Beetles of this family are often associated with birds' nests in the open, but only in the moister of the dry nests, usually in those of pigeons. Little is known of their habits, but they are probably predatory. All those named below have been found in nests and are also associated with stored products under damp conditions—*Gnathoncus rotundatus* (Kuge.), *Dendrophilus punctatus* (Herbst), *Carcinops quattuordecimstriata* (Steph.) and *Hister merdarius* Hoff.

Diptera.

SCENOPINIDAE.

Scenopinus fenestralis (L.). Larvae of the Window Fly are second in importance only to *Lyctocoris* as both nest and warehouse predators.

Hymenoptera.

BRACONIDAE.

Apanteles carpatus (Say). This species is a common parasite of the case-bearing Tineid moth larvae (see Woodroffe & Southgate (1951b)).

Orthostigma pumilum (Nees). This Braconid also attacks the *Tinea* larvae, but only rarely.

ICHNEUMONIDAE.

Stilpnus blandus Grav. A specimen was bred from a larva of *Fannia canicularis*.

Metacoelus mansuetor (Grav.). This species is the commonest parasite of the Tineid case-bearers (see Woodroffe & Southgate (1951b)).

PTEROMALIDAE.

Mormoniella vitripennis (Walk.). This has already been referred to as a common parasite of *Protocalliphora azurea*, one of the bird ectoparasites.

Dibrachys cavus (Walk.). This is a cosmopolitan parasite with a wide range of hosts. It was bred in some numbers from two puparia of the Hippoboscid, *Crataerina pallida*, and, on one occasion, from the case-bearing larvae of the moth, *Tinea columbariella*.

THE MITE FAUNA.

No claim to have surveyed the mite fauna of dry nests with any great degree of thoroughness is made. The vast numbers of mites which normally occur preclude the examination of all but a very small proportion of the whole. Many species or even genera are indistinguishable unless cleared, mounted and examined under the high power of the microscope. Also, in many groups, identification cannot reliably be carried below the level of family. Consequently it is probable that many species have been overlooked, especially if they were present in small numbers among much larger numbers of a closely similar species.

(a) Ectoparasites of Birds.

Parasitiformes.

LAELAPTIDAE.

Dermanyssus gallinae (Deg.). This is the common fowl mite and it was present in most nests, often in very large numbers. It may, in its nymphal stages, serve as prey for predatory species but otherwise has no importance in the nest fauna.

(b) Scavengers.

Sarcoptiformes.

TYROGLYPHIDAE.

Tyroglyphus farinae (Deg.). The Flour Mite is the most important mite pest of stored cereal products. It occurs in nests with moderate frequency and reaches high population densities in some pigeons' nests. The record below indicates its occurrence in areas remote from buildings.

Record. Shelduck, on ground under hawthorn bush (*Crataegus*) in meadow, East Bergholt, Suffolk, 5 Oct. 50, MN.

Tyrophagus tenuiclavus Zach. This species is somewhat less important than *Tyroglyphus farinae*, and is found principally on materials with a high protein or fat content (Hughes, 1948). It is, however, more abundant in nests, and is often the dominant mite species in pigeons' nests.

Tyrolichus casei Ouds. *T. casei* is similar in its food preferences to *Tyrophagus tenuiclavus*, but is of less importance as a pest. It was found occasionally in small numbers in nests.

Thyreophagus entomophagus Lab. This mite has been found in small numbers in nests on several occasions. It has been reported from stored cereal products and is known to damage insect collections (Hughes, 1948).

Mealia sp. Previously, *Mealia pteronyssina* Berl. has been recorded as one of the dominant nest species. Recently, however, specimens have been submitted to Dr. Cooreman of the Royal Belgian Natural History Museum, Brussels, an authority on the genus, and he is of the opinion that they are of a new species which he has kindly agreed to describe. It occurs in very large numbers in most nests, being usually dominant in sparrows' nests. It appears to be less susceptible to low humidities than the other Tyroglyphids mentioned and was found in some very dry swallows' nests which were otherwise practically devoid of mites.

GLYCYPHAGIDAE.

Glycyphagus domesticus (Deg.). This is one of the commonest of the stored-products and domestic mites. It occurs on almost any material in a variety of situations, but has little economic importance. It is more widespread and abundant in nests in general than any other species.

Glycyphagus ornatus Kram. This species has occasionally been detected among the *G. domesticus* population. Probably it occurred frequently and was usually overlooked.

Glycyphagus n. sp. On several occasions a *Glycyphagus* was found which did not appear to correspond with any of the species described by Hughes (1948). It was conspicuous because of the bright red colour of the lateral vesicles, a feature which appeared to be constant and which was present in all developmental stages. Miss P. L. Robertson, then working at this Laboratory, was of the opinion that it was a new species and her description of it is in preparation. She will also give detailed records of its occurrence.

Otenoglyphus plumiger Koch.

Otenoglyphus canestrinii Arm. These two species have occasionally been found in nests. They occur also in food-storage premises, but are of no importance as pests.

(c) Predators.

Of the predatory mites which have been found in nests, only the Cheyletids are of any great importance. The others are chiefly casual predators without any real association with nests.

Because of the difficulty of identification, the "Gamasids" have been treated as a group (although the genus *Typhlodromus* has been repeatedly determined) and, as a group, they have some small significance as general predators in warehouses.

Parasitiformes.**LAELAPTIDAE.**

Typhlodromus sp.

Trombidiformes.**BDELLIDAE.**

Bdella sp.

TYDEIDAE.

Tydeus sp.

CHEYLETIDAE.

Acaropsis docta (Berl.). This Cheyletid occurs occasionally in stored products. It was found in large numbers in sparrows' nests, and less abundantly in those of the other birds. Its predatory habits will be discussed later with those of the other Cheyletids.

Cheletomorpha venustissima (Koch). A species which is occasionally found on Tyroglyphid-infested products, this predator was found occasionally, and in small numbers, in nests.

Cheyletia flabellifera (Mich.). *C. flabellifera* occurs occasionally among the other Cheyletids in nests and was found in some numbers in those that contained much green moss. It has been reported in small numbers on stored products.

Cheyletus eruditus (Schr.). This is the commonest predatory mite found associated with Tyroglyphids on stored products. It is widely distributed in nests, often only in small numbers, but was abundant in most pigeons' nests.

Cheyletus sp. On one occasion a house-martins' nest from Bedford was found to be swarming with large Cheyletids which were engorged with blood. It has so far proved impossible to determine the species.

CUNAXIDAE.

Cunaxa capreolus (Berl.). This and the next species were found frequently, though in small numbers, particularly in sparrows' nests.

Cunaxa setirostris (Herm.).

TROMBIDIIDAE.

Trombidium sp.

PSEUDOSCORPIONS.

Chernes sp. occurred frequently in nests, often in very large numbers.

Chelifer cancroides (L.) was abundant in some pigeons' nests.

Both were probably predatory upon mites or the young larval stages of insects.

THE ECOLOGY OF THE NEST FAUNA.

The Dry Nest Habitat.

PHYSICAL CONDITIONS.

Humidity conditions.

A bird's nest that is subject to saturation by water undergoes rapid bacterial and fungal decomposition and has a fauna similar to that of decaying vegetable matter in a wide variety of situations. Nests built in the open show these features after they have been abandoned by the birds. On the other hand, a nest that is protected from rain or drainage water decomposes comparatively slowly, and the scavenging fauna of insects and mites that it supports differs widely from that of the wet, exposed type. Nests that are usually dry include those of the house-sparrow (*Passer domesticus*), house-martin (*Delichon urbica*), swallow (*Hirundo rustica*), swift (*Apus apus*), starling (*Sturnus vulgaris*), jackdaw (*Corvus monedula*), and city pigeon (*Columba* sp.). It must, however, be emphasised that it is the position of the nest, and not the species of bird that determines the type. Of the birds named, most habitually build in sheltered situations, but when a house-sparrow, for example, builds in a tree, the nest, with its scavenging fauna, is of the wet type. Similarly, the insect and mite fauna of a blackbird's nest, which is normally of the wet type, includes many dry-nest species if the bird builds its nest in a shed. It is greatly to be regretted that almost all the records of insects taken from birds' nests, while including information as to bird species, make no mention of the position or condition of the nest. Dry examples of nests of the following birds that normally build their nests in exposed situations have been examined: blackbird (*Turdus merula*), robin (*Erithacus rubecula*), spotted flycatcher (*Muscicapa striata*), blue tit (*Parus caeruleus*), tree-creeper (*Certhia familiaris*), wren (*Troglodytes troglodytes*), redstart (*Phoenicurus phoenicurus*), pied wagtail (*Motacilla yarrellii*), and shelduck (*Tadorna tadorna*). It is impossible to draw a precise line between the two nest types. In many nests intermediate conditions are found, and here a mixture of the more tolerant insect species of both groups occurs. Also, some species (e.g., *Monopis rusticella*) are found in both of the extreme types, although, when this occurs, there is usually a marked preference for one or the other.

Finally, it must be emphasised that the terms "wet" and "dry" are relative only. The relative humidity within a nest may be constantly near saturation, but if liquid water is never present in appreciable quantities the nest is of the dry type.

Temperature conditions.

It seems unlikely that temperature has any decisive effect upon the nest fauna. While the nest is in use, particularly when the brood is present, its temperature will be considerably higher than that of its surroundings, and there will be no nocturnal fall. These conditions will enable many species to develop rapidly, but once the nest has been abandoned by the birds, temperature conditions will revert to normal, and only those species that can overwinter in the open will survive. It is possible that less hardy species could survive in nests of sparrows and pigeons as these birds often use their nests for roosting throughout the year. In these nests, also, species without a winter diapause would be capable of fairly rapid development during the winter.

THE COMPOSITION OF THE NESTS.

The nest materials comprise organic matter of both animal and vegetable origin. Those of the exposed nest, once it has been abandoned by the birds, are subject to alternate desiccation by sun and wind and saturation by rain. Under these conditions the nest is rapidly reduced to a mass of humus bound together by the coarser fibrous materials. Very soon most of the finer material is washed out by rain, and only the fibre remains. On the other hand, the materials that compose a sheltered nest decompose so slowly that they persist in their original condition for considerable periods and can consequently form a source of food for a more or less permanent population of those insects and mites that are able to thrive on dried organic materials.

The Dry Nest Community.

It is convenient to make an ecological classification of the insects and mites that breed in nests by considering their methods of obtaining food.

The ectoparasites of the birds are entirely dependent upon them for food and they are of importance in the nest fauna only in so far as they form the prey of predatory species.

The scavengers, with which are included the mycetophagous species, form the largest and most important group. They feed upon the nest materials, the excrement and other waste products of the birds, or upon moulds growing on these materials. The larvae of fleas must be considered as belonging to this group although the adults are entirely ectoparasitic.

The third group consists of insects and mites that are predatory or parasitic upon other nest inhabitants, and this class includes the parasitic Hymenoptera.

If the bird ectoparasites are ignored, the dry-nest fauna consists of scavengers of dried organic materials and their natural enemies. This community can be divided into three groups according to the status in the nest. Group I consists of typical nest-dwelling species. Group II comprises species that are occasional nest-dwellers; that is, they occur infrequently, but may breed successfully if they are able to reach the nest, or when particular conditions prevail. Group III includes the casual visitors, species whose status is doubtful, and those of wide distribution that inhabit the nests only incidentally as an extension or a part of their usual habitat. This classification of species is given in Table II.

The species in Group I are those that truly characterise the dry-nest community. Such a group of species occurs in no other natural habitat in this country, although fragments of it occur in such situations as the nests of rodents

Classification of dry-nest species according to status.

		GROUP I Regular inhabitants	GROUP II Occasional inhabitants	GROUP III Incidental inhabitants, casual visitors, and species of doubtful status
Insects— Scavengers— Various orders	..	<i>Hofmannophila pseudospretella</i> <i>Endrosia sarcitella</i> <i>Tinea pellionella</i> <i>Tinea columbariella</i>	<i>Lepisma saccharina</i>	Collembola Psocids Isopods Dermoptera
	Lepidoptera ..		<i>Monopis crociapitella</i> <i>Tinea pallescens</i>	<i>Ephestia elutella</i> <i>Pyralis farinalis</i> <i>Monopis rusticella</i> <i>Monopis weaverella</i> <i>Tineola bisselliella</i> <i>Tinea fuscipunctella</i>
Coleoptera	..	<i>Attagenus pellio</i> <i>Anthrenus verbasci</i> <i>Pinus fur</i> <i>Pinus tectus</i> <i>Tenebrio molitor</i>	<i>Dermestes lardarius</i> <i>Megatoma undata</i> <i>Anthrenus fuscus</i> <i>Anthrenus muscorum</i> <i>Stegobium paniceum</i> <i>Pinus pusillus</i> <i>Pinus secpunctatus</i>	<i>Oryzaephilus surinamensis</i> <i>Ahasverus advena</i> Cryptaphagidae Lathridiidae Other Ptinidae <i>Tenebrio obscurus</i> <i>Trox scaber</i>
Diptera	..	<i>Fannia canicularis</i>	<i>Calliphora erythrocephala</i> <i>Sarcophaga barbata</i>	<i>Tephrochlamys tarsalis</i> Other Diptera
Predators	<i>Lyctocoris campestris</i> <i>Scenopinus fenestralis</i> <i>Apanteles carpatus</i> <i>Metacoelus mansuetor</i> <i>Mormoniella vitripennis</i>	<i>Empicoris culiciformis</i>	<i>Reduvius personatus</i> <i>Orius majusculus</i> Staphylinidae Carabidae Histeridae Other Hymenoptera
Mites— Scavengers	<i>Tyroglyphus farinae</i> <i>Tyrophagus tenuiclavus</i> <i>Mealia</i> sp. <i>Glycyphagus domesticus</i>	<i>Tyrolichus casei</i> <i>Glycyphagus ornatus</i> <i>Glycyphagus</i> sp. <i>Ctenoglyphus</i> spp.	<i>Thyreophagus entomophagus</i>
Predators	<i>Acaropsis docta</i> <i>Cheyletus eruditus</i>	<i>Typhlodromus</i> sp. Tydeidae <i>Cheletomorpha venustissima</i> <i>Cheyletia flabellifera</i> <i>Cunata</i> spp.	Other Gamasids <i>Badella</i> sp. Trombididae
Other Arthropoda	..		Pseudoscorpions	Spiders Centipedes

K

and social insects and beneath the bark of trees. In unexploited countryside the distribution of the community corresponds with the distribution of the dried organic material upon which it subsists, and this is scanty and discontinuous, forming small, widely separated pockets. Except when they are associated with buildings, birds' nests in our climate are rarely dry. Even when situated in holes in trees, the nests tend to be intermediate in type, some part usually being affected by seepage of rain water. Probably only house-martins' nests, situated under cliff ledges, and the nests of swifts and jackdaws, in crevices and fissures in the rock, are sufficiently protected to provide, occasionally, a really dry nest. Before this community was affected by human activity many of its constituent species must have been rare insects, and this is still true of the entirely rural parts of the country. In his "Preliminary List of the Coleoptera of Windsor Forest" (1939), Donisthorpe says of *Attagenus pello*: "In cut grass; very scarce." (The cutting of the grass was presumably a human activity.) Of *Anthrenus verbasci* he remarks that the species was taken "once, by sweeping flowers". He does not record *Ptinus fur* or *Dermestes lardarius* at all, and records *Tenebrio molitor* only in association with buildings. The effect of urban development upon this local and closely circumscribed community has been profound. The buildings of our towns and farms provide dry nesting sites for a vast population of birds, and this considerable food supply is supplemented by the dried organic matter, in the form of stored food and clothing, which is contained within the buildings. Some buildings are used solely for the purpose of storing such materials, and the development of large towns ensures that the available nesting sites and supplementary food supplies are in close proximity. It is not surprising, therefore, that in built-up areas these originally rare dry-nest species are now common insects. Some of them have become serious pests, and most are now regarded as "indoor" species. In undeveloped rural areas the dry-nest community still exists in small, isolated pockets, but in those parts affected by the builder, the house, warehouse, barn or even the whole town, may be regarded as the unit which the original dry-nest community now occupies.

The Influence of Bird Species upon Nest Fauna.

The basic materials used for nest construction by the various birds studied in the course of this work are very similar as regards suitability as food for scavenging insects. The nests differ, however, in the proportion of the various materials present, and in certain other important characteristics such as humidity, and these differences are reflected in the composition of the fauna of insects and mites. The following paragraphs indicate the particular features of the nests of each of the more important species of bird that significantly affect the composition of the scavenging population.

House-sparrow (Plate XIV, fig. 1).

The house-sparrow's nest has been selected as a typical dry nest, and forms a standard for comparison with other species. It consists of a mixture of vegetable fibre (straw and dry grass), animal material (horse-hair, feathers and excrement) and a fine dust the origin of part of which is obscure, but which includes a proportion of insect faecal pellets. The dust is probably important for mites and the very young stages of insects. Such nests are moderately dry and are usually dominated by the case-bearing Tineid moth, *Tinea columbariella*. The Oecophorids, *Hofmannophila pseudospretella* and *Endrosis sarcitrella*, are also abundant, the former more so than the latter. Dermestid, Ptinid and Tenebrionid beetles are present in moderate numbers. Among the scavenging mites, *Mealia* sp. is dominant, with *Glycyphagus domesticus* abundant and *Tyroglyphus farinae* and *Tyrophagus tenuiclavus* in moderate numbers.

House-martin (Plate XV, fig. 2).

The house-martin's nest consists of a mud cup, lined with feathers and dry grass, usually placed close beneath some over-hanging ledge so that the entrance consists of a small hole. The quantity of nest material is small, and the total number of insects found is correspondingly smaller than in the sparrow's nest. The chief difference between the two is that the mud cup usually maintains a higher humidity in the nest of the house-martin than is found in the loosely constructed nest of the sparrow. Consequently, *Hofmannophila* is usually the dominant insect and *Glycyphagus domesticus* or *Mealia* the dominant mite. The *Hofmannophila* larvae certainly burrow into, and appear to feed on, the mud cup as well as the lining. This is probably responsible for the breakdown of many of the nests during the winter.

City pigeon (Plate XIV, fig. 2).

The typical city pigeon's nest is formed by a slight depression in a mound of guano with which are incorporated a few feathers and pieces of straw. Often large nesting colonies are formed and used for many years. When this occurs the quantity of material present is extremely large. The important factors, in addition to the bulk of the utilisable material, are its very solid nature, its almost exclusively animal origin and the high humidity, particularly within the larger masses of guano. Typically, dominance is shared by *Hofmannophila*, *Ptinus tectus*, *Stegobium paniceum* and *Tenebrio molitor*, with *Lepisma saccharina* and *Fannia canicularis* usually abundant. *Tinea pellionella* and *Dermestes lardarius* are often present in moderate numbers, but *Anthrenus* spp. are seldom found. Mycetophagous species (e.g., *Enicmus*, *Cryptophagus*) occur more frequently here than in nests of other species. *Tyrophagus tenuiclavus* and *Tyroglyphus farinae* are the dominant mites with *Glycyphagus domesticus* abundant and *Mealia* sp. comparatively scarce. It is noteworthy that *Dermestes*, *Stegobium*, *Fannia* and *Lepisma* have rarely been found in other nests and never in comparable numbers.

Jackdaw.

The nest is composed of twigs, with the nest cup lined with dry grass, sheep's wool, pieces of paper and string, and dry rubbish of many kinds. Excrement is absent and there is usually a great quantity of dust. The nest may be situated in a recess in masonry or in a hollow tree. The population of insects and mites is usually sparser than in the nests of the previous species. The most conspicuous insects are *Hofmannophila* and *Attagenus pello* with *Endrosis*, *Tinea pellionella* and *T. columbariella* abundant and *Ptinus fur* and *Lepisma saccharina* in moderate numbers. *Glycyphagus domesticus* is usually the most abundant mite, with *Mealia*, *Tyroglyphus* and *Tyrophagus* in smaller numbers.

Swallow (Plate XV, fig. 1).

The open mud nests of the swallow are usually only scantily lined with dry grass, or occasionally feathers. They are situated on beams in barns or other buildings and are usually very dry, sometimes containing practically no life. The Dermestids, *Anthrenus verbasci* and *Attagenus pello*, sometimes reach moderate numbers; *Tinea columbariella* is often present, and so is *Hofmannophila* when humidity is high enough. But, in general, swallows' nests cannot compare with those previously described as favourable habitats for insect life. *Glycyphagus domesticus*, *Mealia* and *Tyrophagus* all occur, but seldom in large numbers.

Predator-Prey Relationships.

A number of the casual visitors listed in Group III of Table III are predators. They seldom show a marked preference for any particular prey, and exert no significant influence on the nest population. On the other hand, the predators in Group I form an important part of the nest community and, from casual observations made during the examination of nest material, it is possible to give

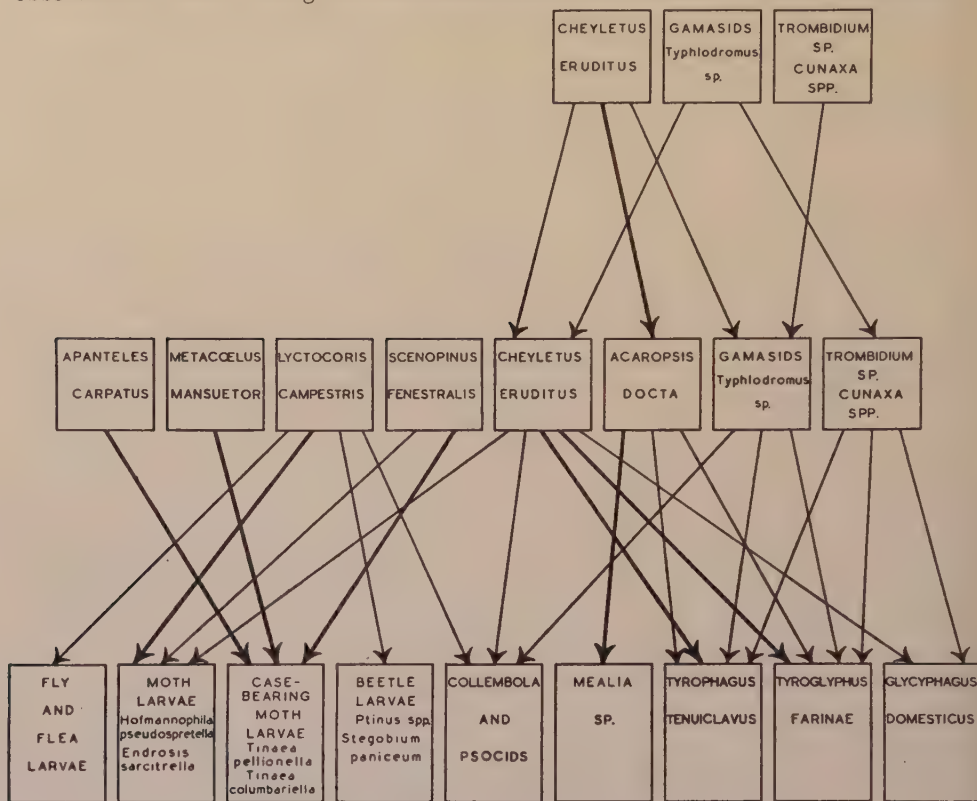


Fig. 2.—Some predator-prey relations within the dry-nest community.

some account of their habits and preferences. Figure 2 illustrates the predator-prey relationships that have been observed. The thick arrows indicate the preferred species of prey when several are available.

Lyctocoris campestris.

This is the only predator which has been observed to affect the density of the scavenging population. On several occasions, extreme abundance of this species has been associated with a rather low level of occurrence of the dominant Lepidoptera. The adults and larger nymphs have frequently been observed to feed on *Hofmannophila* and *Endrosis* larvae (see Pl. XVI, fig. 2), and, more rarely, on Ptinid larvae, Collembola, Psocids and fly and flea larvae. Smaller nymphs probably feed on young Lepidopterous larvae and possibly on mites.

Scenopinus fenestralis.

The long, thread-like larvae of this fly specialise in attacking the case-bearing Tineid larvae. The Scenopinid larva inserts its head into one end of the case of the

Tinea larva to make its attack (see Plate XVI, fig. 1), so taking advantage of the fact that these larvae are usually very reluctant to leave their cases. This habit renders them very susceptible to the attacks of *Scenopinus*. In pigeon nests the case-bearers are often few in number, but the hard lumps of guano are usually riddled with passages made by Ptinid and *Stegobium* larvae. The *Scenopinus* larvae are very suitably shaped for exploring these burrows, and, in these nests, the beetle larvae are probably their chief prey. *Scenopinus* also occasionally attacks the House-Moth larvae. A full-grown *Hofmannophila* larva possesses powerful mandibles which it is not reluctant to use. An attack by *Scenopinus* on a *Hofmannophila* larva which was able to defend itself would probably end in the death of the attacker. However, a successful attack was observed on a larva that had spun up for diapause or pupation. *Endrosis* larvae are less aggressive and are probably more frequent victims.

Acaropsis docta and *Cheyletus eruditus*.

The dominant scavenging mite species in sparrows' nests are usually *Mealia* sp. and *Glycyphagus domesticus*. Because of its long setae and rapid movements the latter is not preyed upon to any great extent by Cheyletids. Consequently, *Mealia* must form the staple diet of predators in these nests, and this fact appears to determine the relative abundance of *Acaropsis* and *Cheyletus*. Usually the former is very abundant and the latter comparatively scarce. The explanation seems to lie in the different hunting methods of the two predators. *Cheyletus* prefers to back into some crevice, where it waits, with pedipalps in the attacking position, for something to move within range. It snaps automatically at any moving object, and, unless the attack is successful, runs rapidly backwards and finds another crevice. In contrast to this, it has been observed that the smaller and less aggressive *Acaropsis* uses its pedipalps to examine and turn over small objects. *Mealia* is a small and sluggish mite and *Cheyletus* seldom seems to be aware of its presence, but with its more appropriate method of hunting, *Acaropsis* is able to deal with a prey of this type. Thus, the usual position is that *Acaropsis* preys upon *Mealia* and *Cheyletus* upon *Acaropsis*.

The position is rather different when *Tyroglyphus* or *Tyrophagus* is present. These species are suitable prey for *Cheyletus*, which is consequently very abundant, a state of affairs often found in pigeons' nests. The determining factor is probably humidity. *Tyroglyphus* and *Tyrophagus* require a higher humidity than either *Mealia* or the Cheyletids. A fall in humidity which adversely affected the *Tyroglyphus* would compel the *Cheyletus* to feed upon its own species, and, provided that sufficient *Mealia* were present, the *Cheyletus* would decrease and the *Acaropsis* increase until the position resembled that usual in sparrows' nests.

Gamasids.

Predators of the *Typhlodromus* type are often present in moderate numbers in nests. They are usually indiscriminate in their attacks and seem rarely to influence the Tyroglyphid-Cheyletid relationship to any great extent. The outcome of a *Typhlodromus*-*Cheyletus* battle varies, but the advantage lies most often with the Cheyletid.

Colonisation of Nests.

One of the major problems facing the nest-inhabiting species is that of reaching the nest. Although many of the nests that are being considered in this paper are used repeatedly, and may last a number of years, the habitat is essentially ephemeral, and reinfestation from outside must be frequently necessary. In many species the adults normally leave the nest, or the larvae

wander away from it before pupation, and consequently reinfestation from outside must normally occur in these species.

Since some species occur in a very high proportion of the nests examined, it seems unlikely that their presence can be entirely fortuitous in all cases. This is particularly true of those nests that possess a small entrance hole. Some mechanism must exist which ensures that a proportion of individuals of these species reach suitable nests. Consideration will now be given to the possible methods by which this may be achieved. Although there is not much positive information, there is a certain amount of evidence upon which tentative suggestions can be based.

Attraction of flying adults.

There is some evidence that certain species, the adults of which are capable of flight, possess a definite behaviour pattern, probably based on an olfactory reaction, which enables them to find a nest. A preliminary experiment has been carried out, in conjunction with Mrs. G. M. Blake, of this Laboratory, who is studying the behaviour of *Anthrenus verbasci*. Boxes containing sterilised nest material were erected in pairs on the walls of one of the Laboratory buildings. One box of each pair was protected from crawling insects by a sticky band. All were wired against entry by birds. The nest material was examined at the end of the first summer, and *Hofmannophila*, *Tinea* and *Anthrenus* larvae were present, both in the banded and in the unprotected boxes. A few *Anthrenus* adults were found trapped in the sticky bands. These observations indicate that flying adults of these three species may be attracted to nests. Infestation occurred during the first summer, so the reaction appears to be capable of accounting for a regular annual infestation. These same species were also present in a starlings' nest in a new nesting box at the end of the first season of its use.

In this connection, it is perhaps worth while recording an observation in connection with *Ptinus fur*, a species which, in addition to being widely distributed in birds' nests, is known to breed in nests of bees and wasps (Linsley, 1944). This Ptinid has been observed to fly only very rarely, and such flight as has been observed has usually been more of a controlled fall than "purposeful" flight. The only occasion on which the writer has observed *P. fur* in flight was when a specimen entered the open laboratory window in horizontal flight and alighted on the bench. The time was 9.30 a.m. on a cold but sunny autumn morning when it was surprising to see any species of beetle on the wing. During the previous afternoon, a wasps' nest had been collected for examination, and it was left in the laboratory overnight. The room smelt strongly of it when the window was opened in the morning. This isolated observation cannot be taken as conclusive evidence, but it is certainly suggestive, and it may link up with other similar observations on the olfactory reactions of this species.

Attraction of crawling adults or larvae.

This is probably the commonest mode of access of those species that cannot fly. It is likely that the Ptinid beetles, with the exception of *P. sexpunctatus* and possibly *P. fur*, depend upon their climbing powers for reaching nests. It will be seen from Table I that none of the Ptinids occurs with a frequency comparable with that of some of the moths, which fly very much more freely, except for *P. tectus* in pigeon nests, which is a special case and will be discussed later. *P. sexpunctatus*, the only Ptinid which flies at all freely in this country, has, except in one instance, been found only as an occasional individual. It seems unlikely, therefore, that the power of flight alone is a critical factor in determining the distribution of Ptinids in nests. It is possible that *P. sexpunctatus*, which is usually associated with bees, reaches the nests, but finds

the habitat unsuitable; the only available evidence, however, is against this possibility, for *P. seaxpunctatus* was present in considerable numbers, and was, in fact, the dominant species of beetle, in eight nests of the house-martin and five of the sparrow from two widely separated localities in Bedford.

The case of *Ptinus tectus* referred to above is one of particular interest. It has been found in 72 per cent. of pigeon nests, where it is usually one of the dominant species. It has been found also in sparrows' nests, often where there appeared to be no obvious source of the infestation. It cannot fly, and was introduced into this country only about fifty years ago. Undoubtedly this is a case where a species was introduced originally into the buildings and has subsequently spread to the nests. Exchange in both directions has probably facilitated the spread of the species. It is generally true that nests in buildings possess a more numerous and varied fauna than dry nests in more isolated situations, and this is doubtless due to the repeated exchange of species between nest and building, each forming a reservoir from which the other may be reinfested. For some species, such as *Anthrenus verbasci*, sparrows' nests probably have a considerable importance as sources of household infestation.

It is instructive to compare the case of the Common Clothes Moth, *Tineola bisselliella*, with that of *Ptinus tectus*. *T. bisselliella* appears, for some reason, to be unable to exploit the nest habitat effectively. In spite of its almost universal distribution in buildings in this country, it has been found in nests only occasionally, and in small numbers, and in most cases there was a very obvious source from which larvae might have crawled. The difficulty appears to be one of access, for the larvae found have been reared successfully on the nest material. In fact these larvae, and the adults reared from them, have been exceptionally large. The indications are of a lack of a suitable behaviour pattern which will enable the adult to reach the nest.

The occasional occurrence of larvae of such species as *Ephestia elutella* in nests can almost certainly be explained by the supposition that they have wandered from some infestation on grain nearby. They have never been found except in very small numbers.

Conveyance as food by insectivorous birds.

The possibility cannot be ignored that adult insects of a suitable species may occasionally be carried to the nest as food for the young, and subsequently escape and produce viable eggs. It seems unlikely, however, that such a method of access could have any general importance.

Conveyance on nesting materials.

This method is probably of greater importance than the last, and may significantly influence the nest population in certain cases where nest materials are obtained in quantity from infested situations. The presence of considerable numbers of adults of *Oryzaephilus surinamensis* in sparrow nests on an Essex farm can probably be explained in this way. There was no evidence that this species bred or overwintered successfully in this situation. It is very doubtful if any of the more important nest species depend to any great extent on this method.

Conveyance on the birds themselves.

This mode of gaining access to nests has been suggested to explain the presence of *Ptinus tectus* in situations apparently inaccessible to it. There are no records of the beetle having been found clinging to birds, but it seems reasonable to suppose that it sometimes reaches pigeons' nests in this way. It has been found in pigeon droppings on high ledges on St. Paul's Cathedral, London, and in nests in a loft at the top of one of the smoke towers of the Natural

History Museum building, South Kensington. It is hard to imagine how such a species could reach these situations except by clinging to the pigeons. No nesting material was used in either case, but pigeons frequently feed and roost in granaries where *P. tectus* is often abundant, and this species appears to be well adapted to clinging to the feathers or feet of a large bird.

Methods 4 and 5 are probably adequate to explain the observed frequency of occurrence of mites in nests. These are much more widely distributed than the insects and, because of their small size, the chances of their conveyance by these methods are greater.

A REVIEW OF THE WORK OF NORDBERG (1936).

Summary of the Work.

The original publication comprises an introduction and six chapters and this arrangement will be retained below.

INTRODUCTION.

In his opening statement Nordberg expresses the opinion that previous workers have overstressed the importance of physical factors in determining nest fauna, and have attributed little influence to the host animal.

CHAPTER I. METHODS OF INVESTIGATION.

During the years 1929-33, 422 nests of 56 species of bird were quantitatively examined. Most of the nests were collected in Aland, but some came from the Finnish mainland. There was no selection of material. All nests encountered were examined by means of a Tullgren apparatus. The efficiency of this apparatus was investigated, particularly as regards its lethal effects upon the various groups of arthropods. Enhancement factors, based upon the mortality due to the apparatus, were used in all estimates of population density.

CHAPTER II. THE BIRDS' NEST AS A BIOTOPE.

(i) *The nest types.*

The nests are classified according to position: in marshes and floating on water; on the ground; in the open above the ground; in holes and partly in holes. The nests are also grouped as autophagous (from which the young depart soon after hatching) and insessorial (when the young remain in the nest for a considerable period); as excrement-free or excrement-containing; and as annual or perennial.

(ii) *Description of the construction and building materials of birds' nests.*

Position and details of construction of the nests are described for each bird species. Also, certain relevant details of the life-histories of the various birds are given, *e.g.*, number of broods, period of occupation, etc. The description of building materials is extremely detailed, all lichens and mosses used being identified.

CHAPTER III. SYSTEMATIC LIST OF ARTHROPODS FOUND IN THE NESTS.

The faunal list includes 528 species of arthropods. Each species is classified as ectoparasitic, zoophagous, necrophagous, coprophagous, schizophagous, phytophagous or indifferent, according to its food preferences, and as eucoene, tycho-coene or xenocoene according to its degree of fidelity to the association.

The species are listed in systematic groups and comprise: Isopoda—1; Collembola—21; Dermaptera—1; Psocoptera—13; Mallophaga—26; Hemiptera—6; Neuroptera—1; Coleoptera—116; Lepidoptera—3; Diptera—21; Aphaniptera—23; Hymenoptera—7; Pseudoscorpiones—3; Araneae—11; Parasitiformes—44; Trombidiformes—60; Sarcoptiformes—169.

CHAPTER IV. THE AUTOECOLOGY OF THE NIDICOLES.

Four groups of ecological factors are recognised as affecting the composition of the nest fauna.

(i) *General geographical-climatic factors.* These were not considered.

(ii) *Local climatic factors.* These include temperature, illumination, relative humidity of the air, relative humidity of the nest material and distance above ground.

Temperature is dealt with in considerable detail. It was measured in various types of nest, in various parts of a nest and at different times during the birds' breeding period. An experimental investigation was carried out to determine the temperature preferendum of some of the nidicoles, and this preferendum is correlated with the temperature of the part of the nest in which a species was usually found. The response of the nidicoles to extremes of temperature was also investigated, in particular the temperature at which they left the nest.

Illumination as a factor affecting the nidicoles was also studied experimentally, and the preferendum was determined for a number of species.

The relative humidity of the air in the nests of several bird species was measured, but was considered to be unimportant in its effects upon the nest inhabitants.

The importance of the relative humidity of the nest materials is discussed in some detail, and was also investigated experimentally, the preferendum being determined for a number of species.

The height of the nest above the ground was found not to influence the composition of the nest fauna.

(iii) *Edaphic factors.* The construction of the nest, *e.g.* its texture, and its importance to the nidicoles is considered at a general level.

(iv) *Biotic factors.* These are very briefly considered under three headings—nutritional relations, reproductive relations and relations to enemies.

CHAPTER V. THE SYNECOLOGY OF THE NIDICOLES.

(i) *Distribution of species, individual and volume quantities of nidicoles among nests of different species of birds.*

The density of habitation per unit volume of nest was estimated both as numbers of individuals and as volume-quantities. Nordberg adopts the latter estimate and gives his reasons for doing so. Tables are given showing density of habitation by nidicoles of the various systemic groups of arthropods in nests of all bird species, and in the four nest groups (aquatic, ground, tree and hole nests), estimated by both methods.

(ii) *Distribution of species and volume-quantities of nidicoles among the different nutrition-biological categories.*

In this section the nidicoles are classified according to their feeding habits (as ectoparasites, zoophages, phytophages, etc.). The habits of the different groups are briefly described and tables give volume-quantities of nidicoles, classified in this way, per unit volume of nest for all bird species.

(iii) *Distribution of nidicoles in different layers of the nest.*

Ten nests, belonging to two bird species, were examined in three layers, and the layering of nidicole species is correlated with their temperature and food preferenda.

(iv) *Sociological characteristics of the stocks of nidicoles.*

(a) The constancy of species in the nests of different species of birds and in different groups of nests.

Several conceptions of constancy and methods of estimating it are discussed. The following degrees of constancy were recognised:—

Constant species — present in more than 50 per cent. of nests examined.

Accessory species — present in 25–50 per cent. of nests examined.

Accidental species — present in less than 25 per cent. of nests examined.

Lists of constant, accessory and accidental species of nidicole are given for each bird species and for each of the four nest groups.

(b) The dominance of volume-quantities of the species in nests of different species of birds.

Three degrees of dominance were used:—

Dominant species (Dominanten) — volume more than 5 per cent. of the total volume of nidicoles.

Influent species (Influenten) — volume 2–5 per cent. of the total volume of nidicoles.

Recedent species (Rezedenten) — volume less than 2 per cent. of the total volume of nidicoles.

The dominants and influents are listed for each bird species, and the significance of dominance is briefly discussed.

(c) The fidelity of the association of the nidicole stock of the nests.

The ideas of various workers concerning fidelity of association are given and criticised. The classification adopted consisted of the following groups:—

Eucoene species—animals that belong exclusively to the nidicole stock of birds' nests or are found there in larger numbers than in other stocks.

Tychocoene species—animals often or even regularly found in the nidicole stock of birds' nests, but not in such numbers as in other stocks which they prefer.

Xenocoene species—animals which belong to other stocks and are found only by chance in the nidicole stocks of birds' nests.

The distribution of these coenological groups of nidicoles among the nests of different bird species is given in a table and illustrated by a graph. The relative proportions of the groups in different types of nest is considered in some detail and some general conclusions are drawn.

CHAPTER VI. THE DEVELOPMENT OF THE NIDICOLE STOCKS.

(i) *How the nidicoles reach the nest.*

Four modes of access are suggested—transportation on the nest materials, transportation on the host animal, chance access and deliberate entrance.

The first three methods are mentioned only briefly, but the last was studied experimentally, and the conclusion was reached that some species search actively for the nest and locate it by smell, often over considerable distances.

(ii) *The development of nidicole stocks in nests used for one brood only.*

The time of arrival and departure of the nidicoles is correlated with the breeding cycle of the birds.

(iii) *The development of the stock of nidicoles in perennial nests.*

This was studied by examining samples at intervals during one summer. The samples were taken from a single large jackdaw colony which was regarded as a single homogeneous unit. Graphs are given showing the variation during the summer of the total animal stock and of the relative proportions of species comprising different nutritional categories.

Discussion of Nordberg's Work.

When reviewing a paper of this size it is necessary, for the sake of brevity, that attention should be largely confined to those points where some disagreement occurs. The above summary has indicated the immense scope of the work, which contains much valuable information and some stimulating ideas, and this must be emphasised in view of the largely adverse criticism that follows.

I. METHODS.

The disadvantages of using automatic methods of separation in order to provide a quantitative estimate of density of habitation have already been considered to some extent. Eggs and pupae cannot be detected by such methods, and there are indications that such groups as lepidopterous larvae are affected by the Tullgren apparatus to a greater extent than Nordberg allowed for by his use of enhancement factors. It is interesting to note that his faunal list includes only three species of Lepidoptera, whereas four species of leaf-eating Chrysomelid beetles, whose presence could only have been fortuitous, were recorded. In Britain, microlepidopterous larvae are usually among the dominant inhabitants of dry nests (Table II gives 12 species), while in exposed nests, such species as *Monopis rusticella* and *Tinea ganomella* Treit. occur in considerable numbers and with very high frequency. While these particular species may not occur in Finland, it is very surprising that 422 nests of 56 bird species contained only three species of Lepidoptera. Also, automatic collection involves killing the insects and this often creates considerable problems in identification. The determination, to species level, of lepidopterous, coleopterous or dipterous larvae is a problem which few specialists in those groups would undertake with confidence. Furthermore, such methods automatically eliminate any chance of detecting the presence of hymenopterous or dipterous parasites of the nidicole species.

II. THE BIRDS' NEST AS A BIOTOPE.

Nordberg classifies nests according to position but he fails to recognise the overriding importance of the degree of exposure to rain. The wet-dry classification is fundamental in the study of nest fauna and the position would be simplified in many ways if these two types of nest were regarded as forming two distinct biotopes. Nordberg's annual-perennial grouping approximates fairly closely to the wet-dry classification, but differs from it in that the perennial nest situated in the open should be regarded as annual in character. Such nests are exposed to the winter weather and are either reduced to a mass of humus or the finer materials are washed or blown away, leaving only the framework of coarse materials to form the foundation of the next year's nest. Consequently they are largely rebuilt each year, though on the same site.

III. THE FAUNAL LIST.

While the sheer magnitude of the task of determining accurately such a list of species as Nordberg presents inevitably raises doubts as to the reliability of the more difficult identifications, these must, in the absence of contradictory evidence, be accepted at their face value. Some of the records, especially those of certain ectoparasites, are surprising, but it must be presumed that they reflect existing differences between Britain and Finland.

IV. AUTOECOLOGY.

The experimental methods by which Nordberg analysed the response of certain nidicole species to physical conditions appear to be generally sound, although one or two doubtful points should be noted.

Temperature.

The gradient used to determine the preferendum was 20–50°C. This was almost certainly too high. Deal (1941) has studied the temperature preferendum of 16 species of stored-products insects. He found that the range of variation in the response to a temperature gradient within one species was very wide, and that changes of behaviour occurred as a result of changes in pretreatment in respect of temperature, food, etc. For example, both with and without food, adults of *Ptinus tectus* showed a preference for about 8°C., at which temperature they were active (i.e., they were not trapped in the cold zone), but on one occasion, when tested without food, they showed a weak preference for 20–25°C. Again, adults of *Anthrenus verbasci* showed a peak preferendum below 15°C., but the range extended as high as 30°C. Some species (e.g., *Stegobium paniceum*) gave evidence of two peaks in their range of temperature preference. Very few of these results would have been evident had Nordberg's range of temperature been used, and several of the species tested by Deal are nidicoles. In the face of such variable and conflicting evidence it is possible to conclude only that the insects tested are highly variable in their response to a temperature gradient, or that the experimental methods used by one or both of these workers were insufficiently refined to demonstrate the preferenda accurately.

Humidity.

Nordberg states that he measured relative humidity in the nests of different birds but fails to describe the method used. Also, when studying the effects of extremes of temperature upon the nidicoles, the problem of controlling the relative humidity in the apparatus while the temperature was raised from 0°C. to 60°C. at the rate of 1°C. per two minutes is dismissed by the statement that it was kept unchanged as far as possible. No indication is given of how this was achieved.

A general criticism of this section on autoecology is that several of the most important effects of physical conditions have been overlooked. Throughout his paper Nordberg assumes that nidicole species develop more rapidly and efficiently at moderately high temperatures. This is not universally true. Some species (e.g., *Hofmannophila pseudospretella* and *Anthrenus verbasci*) develop most successfully at comparatively low temperatures. A high temperature may stimulate rapid larval growth but it may also induce a prolonged larval diapause, the final effect being an increased instead of a decreased total development period at the higher temperature. As regards moisture, the most important effect is not the direct effect of humidity upon the nidicole species, but the action of rain upon the nest materials. Nordberg admits that degree of exposure to rain is an important factor controlling the relative humidity of the nest materials, but he fails to realise the primary importance of this factor in determining nest conditions and consequently nest fauna.

V. SYNECOLOGY.

(a) *The advantages of biovolume as an estimate of population density.*

Nordberg adopts the volumetric instead of the numerical estimate of population density because of the considerable size differences between the various species of nest-dwelling arthropods. This method not only takes into consideration the difference in size between an adult mite and an adult insect but also allows for the equally great size difference between a young larval and an adult insect. In the latter instance, it must be remembered that young larvae are potential adults and therefore, in some respects, possess a significance in the population greater than is indicated by their volume. This must obviously be taken into account when considering the degree of dominance of different nidicole

species, and so, for this purpose, biovolume appears to have little advantage over the numerical estimate as a measure of population density. Nordberg's choice of this method indicates what is probably the most important fault in his approach to the problem of the dynamics of the nest population, namely, his static conception of dominance, which permits accurate quantitative analysis but ignores the essentially dynamic conditions within the nest community. This point will be considered in detail later.

(b) *The accuracy of the nutritional and coenological classification.*

Every species in the faunal list is classified according to its feeding habits (*i.e.*, as ectoparasitic, zoophagous, necrophagous, coprophagous, schizophagous, phytophagous or indifferent) and its fidelity to the association (*i.e.*, as eucoene, tychocoene or xenocoene). Nordberg writes, concerning the coenological groups, that in a number of instances it was difficult to make a choice between these categories, as the mode of life of the species concerned was not adequately known. In all doubtful cases the category less well defined was chosen. Not only must this proviso apply equally to the nutritional classification, but, in either case, constitutes a very considerable understatement. Nordberg's list of 528 species of arthropods includes a high proportion of little-known species. In respect of these the apparently preferred habitat is probably the only one which has been sufficiently thoroughly investigated to reveal their presence. In the present state of entomological knowledge it is generally true that the known distribution of a little known species or group reflects the distribution and habits of entomologists rather than of insects. The following examples illustrate some of these points.

The Anthocorid bug, *Lyctocoris campestris*, is a common, widely distributed and well known species. In Britain, it has acquired the common name of "Stack Bug" because of its abundance in haystacks. It occurs also in ditches in open country, on rubbish dumps, in birds' nests, in warehouses, etc. It is predominantly carnivorous in Britain, feeding upon other insects and small arthropods, but it has been known to bite humans. The other British Anthocoridae have similar feeding habits. This typical predator is classified by Weidner (1952) as an ectoparasite of birds, and by Nordberg as a phytophage. If there can be such a wide divergence of opinion concerning the feeding habits of such a common species as *Lyctocoris campestris*, Nordberg's classification of the less common species cannot be considered reliable.

Several further examples may be briefly dealt with. Nordberg's description of all the Staphylinidae he lists as zoophages is an unjustified assumption. One of the species he so describes (*Bledius diota* Schio.) almost certainly feeds largely upon algae (W. O. Steel, in litt.). *Nycteribia* sp. is described as tychocoene. The Nycteribiidae are ectoparasites of bats and Nordberg records *Nycteribia* sp. only from sparrows' nests, of which he examined six. *Cheyletus eruditus* is the commonest predatory mite found on stored products. Nordberg records it from nests of one bird species (*Columba oenas*) but classifies it as eucoene.

(c) *Constancy.*

Very few of the results of the dry-nest survey in Britain are directly comparable with Nordberg's findings in Finland, but the estimates of constancy for nests of certain bird species may be so compared. The information given in fig. 1 may be expressed according to the degrees of constancy used by Nordberg and compared directly with his results for these insects in nests of the same bird species. Such a comparison, involving, on each side, 115 estimates of constancy, shows that complete agreement on an estimate of "constant" occurred only twice—for *Anthrenus verbasci* in sparrows' nests and for *Fannia canicularis* in pigeon nests. Complete disagreement (*i.e.*, an estimate of

"constant" opposed to one of "absent") occurred 36 times. This contrast must be attributed to differences in methods and to actual faunal differences between Britain and Finland.

(d) *Dominance.*

Mention has already been made of Nordberg's static view of dominance. He analysed nests by automatic methods and designated species as dominant, influent or recedent according to the proportion of the total volume of life which each represented. For any one nest this method gives a valid estimate of volume dominance at a point in time, but it is inadmissible to draw any general conclusions regarding dominance from such analyses unless adequate numbers of nests of each bird have been examined at different times of the year. Nidicole species differ widely in the details of their life-histories and the pattern of volume dominance is continually changing. Species developing at different speeds reach their maximum biovolume at different times. Some species have one generation a year and some several; the adults of certain species are short-lived, or leave the nest immediately, while others live a long time and remain and feed in the nest; one species may reach the nest early in the year and another later, or the same species may arrive several times at long intervals. Nordberg's nest analyses give a few cross-sections of a complex and dynamic pattern but they give no indication of the complete picture. He justifies his methods by stating that the nidicoles make use of all available means of existence so speedily that the nest, even when perennial, becomes quantitatively and qualitatively saturated during the first summer. This is not true of perennial nests in this country. Some observations on this point are recorded in Section 5 of this paper (Colonisation of nests: (a) Attraction of flying adults) and further observations of a similar nature have confirmed that comparatively few species reach the nest during the first summer of its existence, and that their numbers are small. It seems unlikely that complete qualitative and quantitative saturation is ever reached in perennial nests which are added to annually because of repeated use by the birds. A degree of unsaturation is almost certain to exist as conditions vary and populations fluctuate.

Manual, qualitative examination of nests at different times of the year can give a truer picture of the dominance relations of the nest fauna. Eggs and young larvae, although insignificant in volume at the time of examination, are an indication of a potential future dominance, while larval skins and empty pupal cases may indicate a dominance which would have been obvious had the nest been examined earlier. Also, the presence of certain species, e.g., the parasitic Hymenoptera, cannot be detected by any method of collection that kills the host larvae.

An additional example of the inadequacy of Nordberg's conception of dominance is provided by his view of the importance of recedent species. Nordberg writes that recedent species were not considered as they are unimportant for the evaluation of the conditions governing dominance. That this is not always true is shown by the following example. The presence in a nest of several hundred eggs of *Hofmannophila pseudospretella* suggests that, had the nest been examined three months later, a hundred or so fully-grown larvae would have been found; this is almost certainly a case of dominance by any method of estimation. But *Hofmannophila* has one important enemy, the predatory mite, *Cheyletus eruditus*, which can successfully attack the very young larvae (Woodroffe, 1951) (see Pl. XVI, fig. 3). If this mite happened to be present in some numbers when the *Hofmannophila* eggs were hatching, few of the larvae would be likely to survive, and the potential dominance would not be realised. *Cheyletus* is never present in such numbers as to raise the species above the recedent level of volume, but it may often be an important factor governing dominance in the nest.

(e) *The dominance of eucoene species.*

At the end of the chapter on synecology, Nordberg states that the pre-dominance of eucoene groups depends upon the degree of specialisation of the ecological conditions. Earlier in the same chapter, in his discussion of dominance, he writes (to quote the translation): "a species specialized (spezialisierte) for the nest of one particular bird species is best suited to live there. The nests are biotopes of a very specialized (spezielle) kind, conditions of warmth, illumination and humidity differing from nest to nest, and the food is often of a very specialized nature too. A species of nidicole which has its optimum under these conditions will develop a greater degree of dominance than another less specialized species". He then goes on to quote the opinion of Vestal that moderately specialised species have a greater chance to dominate, and agrees that this may be true of biotopes which are poorly differentiated ecologically, but insists that it is not true of birds' nests.

These generalisations give rise to three important questions:—

(i) What is meant by highly specialised ecological conditions and highly specialised species?

(ii) Is a birds' nest a biotope of a highly specialised kind?

(iii) Are eucoene species dominant in nests and, if so, how may this dominance be explained?

These three questions will be considered in turn and for this purpose Nordberg's results will be accepted at their face value. His conception of dominance, the reliability of his coenological classification and his failure to distinguish the wet- and dry-nest biotopes have already been criticised. What has been said in connection with these points obviously has considerable bearing upon these questions. It is, however, instructive to discuss them without reference to previous arguments.

(i) Unfortunately Nordberg fails to explain what he means by highly specialised ecological conditions. Some definition must therefore be suggested. Highly specialised ecological conditions would seem to require, as a minimum, restricted variability and a considerable degree of peculiarity to the biotope. Precise measurement of degree of specialisation is not possible, but some assessment may be made by considering each individual factor in connection with these minimum requirements for high specialisation. If this tentative definition is accepted then a highly specialised species will be one which is closely adapted to highly specialised conditions.

(ii) Nordberg mentions warmth, illumination, humidity and food as factors determining the degree of specialisation of the ecological conditions of the nest biotope. It is not easy to see how a habitat which provides sustenance for ectoparasites, zoophages, necrophages, coprophages, schizophages, phytophages and indifferent feeders can be considered highly specialised from the nutritional point of view. Similarly, in a single nest, physical conditions vary in different parts and, within one bird species, accidents of position may produce a very wide range of variation. In the face of these facts it does not seem possible to regard the nest biotope as highly specialised ecologically. It seems more satisfactory to regard a nest as an island habitat, distinct from the surrounding environment, but not necessarily more highly specialised.

(iii) Nordberg's results support his contention that eucoene species pre-dominate in nests and he explains this by correlating the degree of dominance with several rather nebulous factors, *e.g.*, the degree of isolation of the nest or nest group and the degree of specialisation of the ecological conditions. Since it has been argued ((ii) above) that the nest is not a highly specialised habitat, it is necessary to explain the dominance of eucoene species in some other way. For this purpose it is convenient to consider the two nest types, wet and dry, separately.

(a) Wet nests—*i.e.*, annual and exposed perennial nests.

The time available for colonisation of an annual nest is extremely short and in consequence the problem of early access is a vital one for the nidicoles. In this respect ectoparasites have an overwhelming advantage over all other species because they usually arrive in numbers on the host bird, and this one fact may account for their dominance, and therefore a dominance of eucoene species, in annual nests. By the time non-parasitic species reach the nest in any numbers there is insufficient time to allow them to exploit it to any great extent. This explanation of the dominance of eucoene species in annual nests involves no general assumption of a high degree of adaptation to specialised conditions. It is based primarily upon the importance of the time factor where temporary habitats are concerned.

(b) Dry nests—*i.e.*, sheltered perennial nests.

In Britain eucoene species do not predominate in dry nests. The fauna may, in general, be described as specialised in that it consists of species which feed upon dried organic materials, but few could be described as eucoene. Many are well known pests of stored products and are common also in other habitats. Nordberg's conclusion that eucoene species predominate even in perennial nests may reflect a difference between Britain and Finland or it may be due to the several sources of error which have already been discussed. He refers repeatedly throughout his paper to the rapid summer development and winter hibernation of the nidicoles. In dry nests in Britain, the more slowly developing species reach their peak biovolume during the winter. Nidicoles may arrive at the nest at any time between April and September and development of the offspring of the late arrivals takes place slowly during the winter. Nordberg gives no indication that he is aware of the considerable winter populations of many perennial nests. In his last chapter he describes his investigations into the development of the nidicole fauna. He took samples from a jackdaw colony at intervals and analysed them in his Tullgren apparatus, but these observations were continued for one summer only.

Summary.

The chief aims of the survey and an important conclusion reached during some preliminary work are briefly stated. This latter was the recognition of two distinct nest types—the wet nest, exposed to rain, and the dry nest, sheltered from rain.

The methods used to examine materials and record results are described. They were closely similar to those described in a previous publication.

The insect fauna is listed under three headings: (a) ectoparasites of birds, which includes 12 species; (b) scavengers, including 66 species; and (c) predators, comprising 14 species. Figures are given for the frequency of occurrence and the abundance of the more important nidicole species, abundance being given as an arbitrary estimate. The importance of each as a pest is also briefly stated, and detailed records are given for uncommon or particularly interesting species.

The mite fauna is dealt with in a similar manner. It includes one ectoparasite, 10 scavengers and 11 predators.

The basic composition of nests and the temperature and humidity conditions within them are described briefly and the possible influence of these factors upon the nest fauna is discussed.

The species of the dry nest community are classified, according to their feeding habits, as ectoparasites of birds, scavengers and predators, and according to their status in the nest, as regular, occasional and incidental inhabitants. The distribution of the group of species which truly characterises this community is discussed.

Differences between the fauna of the nests of different bird species are correlated with differences in the composition of the nests. Certain species were found to be particularly associated with certain nests—e.g., *Tinea pellionella* and *Attagenus pelli* with jackdaws, *Anthrenus verbasci* with sparrows and *Dermestes lardarius*, many Ptinidae, *Stegobium paniceum*, *Lepisma saccharina* and *Fannia canicularis* with pigeons.

Details are given of the more important predator-prey relationships which were observed in the nests. The following cases are considered in detail: *Lyctocoris campestris*, predatory upon House-Moth larvae, *Scenopinus fenestralis* upon larvae of the *Tinea* casebearers, and *Acaropsis docta* and *Cheyletus eruditus* upon several Tyroglyphids.

Possible methods of nest colonisation are discussed: (a) attraction of flying adults; (b) attraction of crawling adults or larvae; (c) conveyance as food by insectivorous birds; (d) conveyance on nesting materials; (e) conveyance on the birds. The modes of access of *Anthrenus verbasci*, *Ptinus fur*, *Ptinus seaxpunctatus*, *Ptinus tectus* and *Tineola bisselliella* are considered in some detail.

Nordberg's "Enquiry into the biology and ecology of the nidicoles of birds" is summarised. This work appears to have been overlooked in the past by reviewers of birds' nest entomology.

A detailed discussion is given of the most important points of disagreement between Nordberg's conclusions and those of this paper. Nordberg's work has three chief faults: (a) His quantitative methods were inadequate in some respects, and there are doubts as to the reliability of his information on the feeding habits and distribution of certain species. (b) Certain fundamental ideas, e.g., the importance of bird species in determining nest fauna and his static conception of dominance, appear to be in contrast to the facts. (c) The use, as key factors in his arguments, of conceptions such as "degree of specialization of ecological conditions" which, in the absence of precise definition, are virtually meaningless, and which cannot be measured or easily assessed.

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References.

- DEAL, J. (1941). The temperature preferendum of certain insects.—*J. Anim. Ecol.*, **10**, pp. 323–356.
- FORD, L. T. (1949). A guide to the smaller British Lepidoptera.—230 pp. London, S. Lond. ent. nat. Hist. Soc.
- HINTON, H. E. (1945). A monograph of the beetles associated with stored products. Vol. I.—443 pp. London, Brit. Mus. (Nat. Hist.).
- HUGHES, A. M. (1948). The mites associated with stored food products.—168 pp. London, Minist. Agric. Fish., H.M.S.O.
- LINSLEY, E. G. (1944). Natural sources, habitats and reservoirs of insects associated with stored food products.—*Hilgardia*, **16**, pp. 187–224.
- NORDBERG, S. (1936). Biologisch-ökologische Untersuchungen über die Vogel-nidicolon.—*Acta zool. fenn.*, **21**, pp. 1–168.
- WEIDNER, H. (1952). Die Insekten der Kulturwüste.—*Mitt. hamburg. zool. Mus.*, **51**, pp. 189–173.
- WOODROFFE, G. E. (1950). The identity of the case-bearing clothes moth (Lep., Tineidae).—*Ent. mon. Mag.*, **86**, p. 181.
- WOODROFFE, G. E. (1951). A life-history study of the Brown House Moth, *Hofmannophila pseudospretella* (Staint.) (Lep. Oecophoridae).—*Bull. ent. Res.*, **41**, pp. 529–553.
- WOODROFFE, G. E. & SOUTHGATE, B. J. (1951a). Birds' nests as a source of domestic pests.—*Proc. zool. Soc. Lond.*, **121**, pp. 55–62.
- WOODROFFE, G. E. & SOUTHGATE, B. J. (1951b). A common host and habitat of *Apanteles carpatus* Say (Hym. Braconidae) in Britain.—*Ent. mon. Mag.*, **87**, p. 171.
- WOODROFFE, G. E. & SOUTHGATE, B. J. (1952). *Monopis crocicapitella* (Clem.) (Lep., Tineidae) infesting felt lagging on a water pipe at Harrow, Middlesex.—*Ent. mon. Mag.*, **88**, p. 288.

Since this manuscript was written, Dr. W. J. Hall, of the Commonwealth Institute of Entomology, has drawn my attention to the following recent publication:—

- HICKS, E. A. (1953). Observations on the insect fauna of birds' nests.—*J. Kans. ent. Soc.*, **26**, pp. 11–18.

This paper is of no great importance, but it gives some useful references. In particular, the following two are ecological studies of nest fauna, that of Leleup (1947) being of considerable interest.

- HESELHAUS, S. J. (1915). Weitere Beiträge zur Kenntnis der Nidicolon.—*Tijdschr. Ent.*, **58**, pp. 251–274.
- LELEUP, N. (1947). Contribution à l'étude des Arthropodes nidicoles et micro-cavernicoles de Belgique.—*Bull. Ann. Soc. ent. Belg.*, **83**, pp. 304–343.



FIG. 1. House-sparrow (*Passer domesticus*).

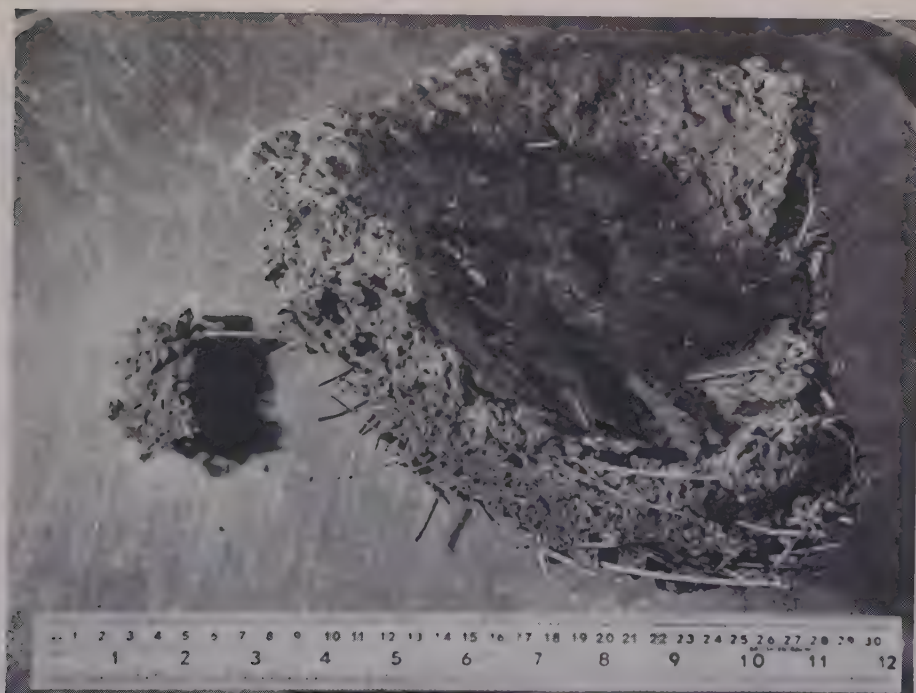


FIG. 2. City pigeon (*Columba* sp.).

SOME TYPICAL DRY NESTS.



FIG. 1. Swallow (*Hirundo rustica*).



FIG. 2. House-martin (*Delichon urbica*).

SOME TYPICAL DRY NESTS.



FIG. 1. Larva of *Scenopinus fenestralis* attacking larva of *Tinaea columbariella*.



FIG. 2. *Lyctocoris campestris* attacking larva of *Endrosis sarcitrella*.



FIG. 3. *Cheyletus eruditus* attacking young larva of *Hofmannophila pseudospretella*.

SOME TYPICAL NEST PREDATORS WITH THEIR PREY.

OBSERVATIONS ON THE BIOLOGY AND MASS-BREEDING OF
SPALANGIA DROSOPHILAE ASHM. (HYMENOPTERA, SPALAN-
 GIIDAE), A PARASITE OF THE FRIT-FLY, *OS CINELLA FRIT* (L.).

By F. J. SIMMONDS.

E.A.N.

Commonwealth Institute of Biological Control.

The general biology of *Spalangia drosophilae* Ashm. has already been described in a paper (Simmonds, 1952), in which this parasite is shown to be able to continue to attack *Oscinella frit* (L.) in the field in Canada after other parasites have completed their activities. It can apparently avoid multiparasitism to a large extent and its efficiency in destroying frit puparia is great. Moreover, it exerts its influence on the host population early in the summer when other parasite species are only beginning to build up their populations. When the complex of parasites attacking the frit-fly in Canada is compared with that in England, it is seen that *S. drosophilae* is the only species without a counterpart there. Hence, this parasite is the most likely to prove effective in England in producing increased parasitism of frit if introduced as a controlling agent.

It was therefore thought desirable to devise a method of producing it in large numbers for shipment and subsequent liberation. As has been indicated previously (Simmonds, 1952), breeding frit-fly in the laboratory on a large scale to obtain unparasitised host puparia for breeding parasites is difficult and impracticable. The collection of sufficient *Spalangia* in the field would be extremely costly. A technique for breeding it on another host (Simmonds, 1944) was therefore necessary. It seemed desirable at the same time to obtain as much detailed information as possible concerning the habits and biological characteristics of the species. For this purpose, extensive experimental work was carried out; an account of some of this work, concerning diapause, has already been published (Simmonds, 1948).

Mass-breeding Technique.

In an attempt to find a host more easily obtainable than *Oscinella*, *Musca domestica* (L.) was first tried, but although oviposition occasionally occurred in puparia of it, it was evidently not suitable. Puparia of *Drosophila melanogaster* Mg. were then tried, and were readily attacked, and it was subsequently found that *Drosophila* was excellent as a host for the parasite. Since this fly can be made available in the laboratory cheaply and in very large numbers, a technique suitable for breeding both host and parasite was developed. As bananas were difficult to obtain at that time, another medium was used for breeding *Drosophila*; it consisted of 86 gm. corn-meal, 370 cc. tomatos (canned), 90 cc. honey and 600 cc. water.

The tomatos are mashed so that few lumps remain (alternatively, tomato juice can be used) and are mixed with water and simmered. The corn-meal is added, and also the honey and the remainder of the water. The mixture has to be constantly stirred to prevent burning. When it begins to thicken it is cooled, and when cool it should be of a fairly stiff, viscous consistency. If it is too fluid, more corn-meal may be added. The above quantities are only approximate, and slight variation of them does not affect the suitability of the medium.

This medium is placed at the bottom of a pint jar to a depth of about an inch, a few drops of living yeast suspension are added, and about 100 *Drosophila* adults are introduced, a cotton wool plug being used as a stopper. The *Drosophila* eggs

can be seen easily on the surface of the medium and on the walls of the jar, and after a few days, when sufficient eggs have been laid, the flies are removed. A piece of paper is placed closely against the glass so as to line the jar, and is left for several days until puparia begin to appear. (Rearing was carried out at 75°F.) The fully grown larvae leave the medium and crawl upwards to a dry place where they form puparia. Only those puparia that are formed on the paper are usable, the puparia on the glass being difficult to remove. The lining papers are changed every day, after the commencement of pupation, but even so, many puparia are formed on the glass and are wasted. To obviate this wastage, the method may be modified; petri dishes 10 cm. in diameter are half-filled with the medium and placed under lamp globes containing flies, and when sufficient eggs have been laid, the dishes are changed and paper is placed between the covering dish and the medium. Nearly all of the puparia are formed on the paper and can therefore be collected. Several hundred to a thousand puparia may be thus obtained from each dish.

For experimental purposes, puparia can best be removed with a camel-hair brush after the papers to which they are fixed have been wetted.

For the mass-breeding of the parasite, it is necessary merely to expose *Drosophila* puparia to ovipositing female parasites in a suitable container. From Table I it is seen that at 75°F. *Spalangia* females lay on the average about 3.4

TABLE I.

Temperature		Length of life of females in days	Postoviposition period	No. of eggs from those females that laid	Sex ratio (from fertilised females only)		Percentage in diapause	Developmental period in days	
					Male	Female		Male	Female
83° F.	Maximum	31	17	183					
	Minimum	13	1	63					
	Average	20.9	4.4	111.1	48.0	52.0	35.6	15.82	17.20
75° F.	Maximum	58	16	226					
	Minimum	15	—	33					
	Average	41.5	5.9	142.8	39.6	60.4	88.3	24.01	25.73
70° F.	Maximum	59	16	160					
	Minimum	15	2	35					
	Average	42.6	6.8	111.5	*61.5	38.5	97.8	26.44	30.50

* Only 21 adults emerged.

eggs per day. It has also been shown that at this temperature superparasitism does not normally occur until 60–70 per cent. of the hosts have been singly parasitised. Thus if 100 puparia are placed with 20–25 female *Spalangia* each day, a parasitism of 60–70 per cent. will be obtained and very few parasite eggs wasted through superparasitism.

Papers bearing *Drosophila* puparia are taken daily or every second day, according to the numbers of puparia being formed, from the host-rearing jars or dishes and placed in a large glass jar. Into this, a suitable number of female *Spalangia* and some males are introduced. To avoid excessive handling, the puparia may be kept in the jars for two or three days and the numbers of parasites put in reduced accordingly. The puparia are then removed and fresh ones are inserted, dead parasites are replaced, and the attacked puparia are set aside for development of the parasites. The unparasitised puparia amongst

these yield adult flies within five days of puparium formation. These flies may be used in the host stock jars. After about three weeks, *Spalangia* adults emerge from the puparia and can be used for further breeding. When sufficient for this purpose have been obtained, the excess can be shipped for liberation or kept in cold storage in the full-grown larval stage. Even in the batches from which the adults are allowed to emerge, there will be a number of full-grown larvae in diapause. These may be stored and later shipped for liberation, as they can be kept without harm in cold storage for lengthy periods.

Thus a routine procedure can be set up that is self-regulatory as regards numbers of hosts and parasites, the extent of it being governed by the numbers of parasites required for shipment and liberation.

Biological Characteristics.

In the course of the study of the life-history of *Spalangia* with *Oscinella* as host, numerous records were obtained of the length of life of the adults, the period of development, fecundity, etc. Before proceeding further with experimental work, it was thought advisable to obtain corresponding but more extensive data with *Drosophila* as host, a breeding technique having been devised such that an adequate supply of host and parasite material was always available.

The work was carried out at 70°, 75°, and 83°F., and the adult parasites were kept isolated in glass vials ($4\frac{1}{2} \times 1$ -in.) from the moment of emergence. The vials were stoppered with a cork on which was a muslin-covered pad of damp cotton wool, to which a split raisin was pinned. In order to obtain data on fecundity and rate of oviposition, individual females were presented each day with 25 *Drosophila* puparia one day old.

With field-collected *Spalangia*, *Oscinella* puparia and similar experimental conditions, including the temperature which was 75°F., females lived on the average 15.4 days (range 1-34 days) and males 18.3 days (range 6-35 days). Isolated females parasitised 55, 58 and 37 frit puparia, respectively, and 20.7 per cent. of the progeny were in diapause.

Data obtained with *Drosophila* as host are given in Table I. They differ greatly from those obtained with *Oscinella* puparia, but it is possible that in the latter case, scarcity of unparasitised frit puparia restricted oviposition, and also that material bred in the laboratory on *Drosophila* for several generations differs from field material in some respects. Mortality in the immature stages was remarkably low—under 3 per cent. The proportion of the progeny of individual females that underwent diapause is interesting, as it differed greatly from that recorded with *Oscinella* as host, even though the conditions of rearing were similar; this has been discussed elsewhere (Simmonds, 1948).

Table II shows the relation between the total number of eggs laid and the number of hosts that are stung but not parasitised. When a large number of eggs is laid, the number of hosts that are stung only is small in comparison with those that are parasitised, whereas when few eggs are laid the number of hosts attacked but not parasitised is comparatively large. This supports the conclusion, based on direct observation, that the acts of stinging and oviposition are two separate processes. Stinging is not necessarily followed by egg-laying; nevertheless, females of low fecundity are capable of killing considerably more hosts than they actually parasitise.

It may be as well at this point to discuss in somewhat greater detail the actual effect of stinging on the individual host, since it is of importance not only in connection with the destructive power of the individual *Spalangia* but also as a factor in the avoidance of superparasitism. After a host has been stung, whether or not oviposition follows, it is paralysed and no further development occurs. An obvious effect is the cessation of heart-beat, but the general effects

of stinging are paralysis, prevention of further development, and the preservation of the host from decomposition for some time.

The importance of the destruction of a number of hosts in excess of those actually parasitised has been emphasised by De Bach (1943). He found that

TABLE II.

No. of eggs laid by individual females	No. of hosts stung only	Total no. of hosts attacked	Percentage stung only
42	44	86	51.2
44	28	72	38.9
49	76	125	60.8
62	63	125	50.4
69	94	163	57.7
105	48	153	31.4
126	30	156	19.2
143	32	175	18.3
158	46	204	22.5
169	21	190	11.1
181	25	205	12.2
210	16	226	7.1

host-feeding, or host-predatism as he terms it, was of great importance in the biological control of the black scale, *Saissetia oleae* (Bern.), by *Metaphycus helvolus* (Comp.), where, particularly at certain densities of host and parasite and in hosts of a certain size, the mortality caused by host-predatism is greater than that caused by actual parasitism. In the present instance, unlike that of the black scale, feeding and oviposition may occur on the same host, and the amount of damage done to the host at feeding is insufficient to render it unsuitable for development of a parasite larva. It would seem that the chain of processes involved in stinging and oviposition is divided into two distinct phases that can be separated after stinging, and that the number of hosts attacked by the female is greater than the number of eggs actually available for oviposition.

The variation in the rate at which eggs are laid throughout the life of the female is of interest, since, if it is considerable, care must be exercised in experimental work to compare oviposition rates only at similar ages. For all the individual females used in the experiment described above, the average rate of egg-laying and attack on hosts at 75°F. is shown in Table III. It was noticed that the trend is the same for both mated and unmated females. A peak in egg-laying is seen to be reached about a week after emergence under these laboratory conditions and subsequently there is a gradual reduction. On the other hand, the number of hosts stung but not parasitised remains fairly constant,

TABLE III.

Age of female in days	0-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45
Eggs laid per female per day (average)	3.37	4.33	3.79	3.12	2.53	2.05	1.62	.83	.65
Hosts stung only (average)	.55	1.15	1.03	1.12	1.17	1.07	1.08	1.02	.42

which again indicates a difference between the potentialities of the stinging instinct and actual fecundity of the females.

Spalangia exhibits arrhenotokous parthenogenesis, like so many other parasitic Hymenoptera. No difficulty was experienced in inducing mating, which occurs very readily on emergence. However, it seemed desirable to ascertain whether a single mating is sufficient to enable the female to produce fertilised (female-producing) eggs throughout life, and how many females a single male can successfully fertilise, in quick succession and at intervals.

The results obtained in the investigation of diapause gave adequate data on the first point. It was evident that at 83°F. and 75°F., spermatozoa remain active in the spermatheca for a maximum of 15–17 days, and at 70°F. for a much longer period, up to 34 days.

While the percentages of the total progeny that are female are approximately the same in the groups developing at 83°F. and 75°F., a greater proportion of females result from eggs laid at the beginning of the female's life at 83°F., and this indicates greater activity of the spermatozoa at the higher temperature even though it is sooner exhausted or dead. At 70°F., spermatozoa are obviously viable for a far longer period than at higher temperatures.

It was thought possible that in each of the temperature groups there might be a difference in the sex-ratio between the progeny developing without diapause and those undergoing diapause. Diapausing individuals kept at 32°F. for about six months were therefore transferred to 75°F. for emergence; it was found, however, that there was no such difference in any group.

These observations suggest that a single mating on emergence is insufficient to ensure the fertilisation of eggs throughout the life of the female, that at higher temperatures the period of activity of the spermatozoa in the females is more restricted, and that the greater activity due to higher temperatures gives a higher percentage of females in the progeny while spermatozoa are available. The percentage of females obtained at 83°F. and 75°F. is consistently lower than that recorded in the field, probably indicating that under natural conditions mating occurs more than once.

In order to obtain some indication of the number of females that can be fertilised by a single male, several fresh virgin females and a single fresh male were used. The females were offered to the male in rapid succession. At first the male mated with the females readily, but after the eighth female, its interest was noticeably lessening and the duration of preliminary courtship and that of copulation were longer. The females were placed separately in vials and given puparia daily as before. The same male was kept until the following day and then offered more females; it paired with three but took no further notice of females. The results of this experiment are shown in Table IV. They indicate that a male is capable of fertilising several females in succession, but that after this, in the same succession, even though mating occurs, no fertilisation takes place. After a period of rest, further successful matings are possible. Under field conditions, therefore, both males and females probably mate more than once.

TABLE IV.

Mating	Day 1										Day 2		
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
Progeny ..	♂♂ & ♀♀	♂♂ & ♀♀	♂♂ & ♀♀	♂♂ & ♀♀	♂♂ only	♂♂ & ♀♀	♂♂ only	♂♂ only	♂♂ only	♂♂ only	♂♂ & ♀♀	♂♂ only	♂♂ only

When these preliminary biological observations had been made, it was possible to continue investigation of *S. drosophilae* in relation to host-selection, superparasitism and diapause. This latter aspect of its biology has already been described (Simmonds, 1948) and the others will be dealt with in subsequent papers. As a final objective, also to be described later, it was hoped that, with this basis of experimental work it would be possible to analyse the development of a population of *Spalangia* in the field and to predict with some degree of accuracy the effect on the frit population in England of the introduction of this species of parasite.

Summary.

A description is given of a technique for breeding the parasite *Spalangia drosophilae* Ashm. (Spalangiidae). Various biological "constants" such as fecundity and longevity were studied at 70°, 75° and 83°F., and the variation in the figures for these is given. The oviposition rate, efficacy of single matings, sex ratio, percentage in diapause, developmental period of both male and female at the different temperatures are also given.

References.

- DE BACH, P. (1943). The importance of host-feeding by adult parasites in the reduction of host populations.—J. econ. Ent., **36**, pp. 647-658.
- SIMMONDS, F. J. (1944). The propagation of insect parasites on unnatural hosts.—Bull. ent. Res., **35**, pp. 219-226.
- SIMMONDS, F. J. (1948). The influence of maternal physiology on the incidence of diapause.—Phil. Trans. (B), **233**, pp. 385-414.
- SIMMONDS, F. J. (1952). Parasites of the Frit-fly, *Oscinella frit* (L.), in Eastern North America.—Bull. ent. Res., **43**, pp. 503-542.
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THE INJURIOUS EFFECTS OF THE HOOKED EPIDERMAL HAIRS
OF FRENCH BEANS (*PHASEOLUS VULGARIS* L.) ON *APHIS*
CRACCIVORA KOCH.

By BRUCE JOHNSON.

E.M.M.

Department of Entomology, Rothamsted Experimental Station.*

It was the practice in Balkan countries to catch bed-bugs on *Phaseolus* leaves spread on the floors of infested rooms (Bogdandy, 1927), and Richardson (1943) found that the insects' legs became impaled on the hooked epidermal hairs. Poos and Smith (1931) had already found that larvae of *Empoasca fabae* (Harris) suffered a high mortality on bean plants by getting caught on the hooks. Larvae of *Leptinotarsa decemlineata* (Say) also become impaled on the hooks and so fail to survive on beans (Trouvelot & Thenard, 1931) and Hely (1945) recorded that adults of the fly, *Strumeta tryoni* Frogg., landing on bean plants became entangled in the hooks and were prevented from taking off. McKinney (1938) thought that the hooks prevented *Myzus persicae* (Sulz.) from becoming established on french beans whilst de Fluiter and Ankersmit (1948) showed that there was a high mortality of *Aphis fabae* Scop. on bean leaves. Schneider (1949) and Miller (1947) claim that the hooks give Aphids and Thrips protection from their predators, thus allowing them to multiply and cause greater damage.

In the present study an attempt is made to evaluate the effect of the hooked hairs on colonies of *Aphis craccivora* Koch on french bean plants in the absence of parasites and predators.

Materials and Methods.

The Aphids used in the experiments were apterous alienicolae from a clone of *Aphis craccivora* Koch started from a viviparous female off cowpeas (*Vigna* sp.) in Sydney, New South Wales, in 1950.

The plants were grown in pots and covered with celluloid and organdie cages. Stock cultures of Aphids were kept on cowpeas and broad beans (*Vicia faba*), and most of the experiments were conducted on the french bean, (*Phaseolus vulgaris*), variety Brown Beauty.

First-instar larvae used in the experiments were obtained by allowing adult Aphids to deposit them where they were required. Where it was necessary to confine Aphids to restricted leaf areas, small celluloid cages half an inch square and with an organdie roof were used. On petioles a celluloid tube was used with organdie sleeves at both ends.

The density of the epidermal hairs was measured by clearing leaves and strips of epidermis from petioles and stems in alcohol and mounting and examining them in water. Strips of leaf 1 cm. wide and running from the base of the leaf to the margin at an angle of 30° from the main vein were taken. The densities given are the approximate average of twenty-five counts in squares each of 0.0038 sq. cm. taken at random along 10 strips.

The Epidermal Hairs.

The small hooked epidermal hairs are scattered over almost the entire surface of french bean plants. Each hair is of three cells (fig. 1), a long thick-walled

* This work was carried out at the Department of Zoology, University of Sydney, New South Wales.

rigid apical cell bent distally to form a strong, sharp-pointed hook, a short stalk cell also with a thick rigid wall, and a basal cell with the wall not so heavily thickened. The whole hair can be twisted and turned freely on its base. The points of the hooks face in all directions and are not all directed the same way as with the climbing hairs of various other plants (Haberlandt, 1914).

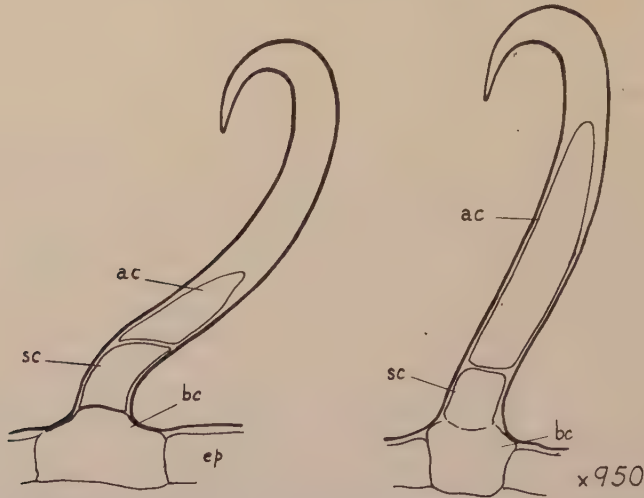


Fig. 1.—Hooked epidermal hairs on french beans. *ac*, apical cell; *sc*, stalk cell; *bc*, basal cell; *ep*, epidermis.

The hairs vary in size on different parts of the plant. Between the veins on the undersurfaces of the leaves they are of fairly uniform length (av. 0.077 mm., range 0.06–0.09 mm.); along the veins of the leaves and on the petioles, stems and fruit they are slightly longer and, except for those on the leaves, the hooks are less well developed; their average length is 0.095 mm. (range 0.084–0.18 mm.).

On the young shoots the hairs are densely packed together and as the leaves expand they become more widely spaced. On the mature parts of the variety Brown Beauty the hairs are most dense on the undersurfaces of the leaves (3,400/sq. cm., range 2,700–4,500) and less dense on the petioles, stems and fruit (1,000/sq. cm., range 500–1,300), while on the upper surfaces of the leaves there are practically no hairs at all. There is no apparent variation in the density of the hairs from the base to the apex of the leaves. On the petioles, the hairs are more dense in the groove running along the upper surface. On the simple primary leaves on some plants of this variety the hairs are dense (3,000/sq. cm., range 2,100–4,000) while on others they are sparse (1,000/sq. cm., range 800–1,400). These will be referred to as primary leaves A and B, respectively.

Hair-density varies with the variety of bean. On the leaves of very hairy varieties, the hair-density is high in the spaces between the veins and lower along the veins, while on sparsely hairy varieties the hairs are sometimes present only along the veins.

Mechanism of Capture.

McKinney (1938) thought that the Aphids became impaled due to the movement of the plants by the wind, and in quick movements “as in attempting to escape parasites and predators.” In the present study Aphids were not usually hooked in this way.

Undisturbed Aphids walking across a leaf move their legs about tentatively, and drag them as they move forward. Feeding Aphids also tend to move their legs about. In both cases, the more susceptible parts of the legs may become impaled on the sharp hooks which, in the majority of cases, penetrate the intersegmental membranes.

Aphids are frequently caught by the tarsal claw; the point of the hook pierces the membrane between the tarsus and pretarsus and between the unguifer and unguitractor plate, and slides down into the cavity of one of the claws (fig. 2 (i)

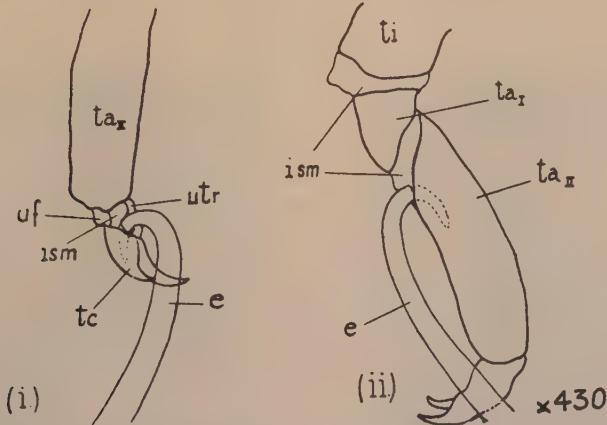


Fig. 2.—Hooks penetrating the intersegmental membranes of Aphids' legs between (i) tarsus and pretarsus; (ii) the two tarsal segments. *e*, epidermal hairs; *ism*, intersegmental membranes; *ta* I, II, tarsal segments 1 and 2; *tc*, tarsal claw; *uf*, unguifer process of tarsus; *utr*, unguitractor plate.

and fig. 3). Hooks also commonly enter the membranes between the tarsal segments and between the tibia and tarsus (fig. 2 (ii) and figs. 4 & 6); sometimes they penetrate the integument of the leg segment (fig. 5).

When an Aphid becomes hooked by the leg, it pulls and moves about driving the hook in more securely. If the pulling is slackened, the leg may slide off the hook and the Aphid escape; frequently, however, struggling continues and the leg may be pulled off where the hook enters. The late instar larvae and adults of a colony are almost invariably found to have part of at least one leg missing, most commonly the tarsal claw; missing tarsal segments are common, and less often the tibia and femur are also torn off. Aphids have been seen with parts missing from five legs. *Aphis craccivora* is incapable of regenerating part of a leg, although it continues to moult.

Aphids are continually getting caught and escaping, and interruptions of feeding, wounds, and loss of blood may therefore result in a lower reproductive rate, reduced size, and increased length of larval development. Struggling to release its leg, an Aphid may get other legs caught on nearby hooks and become securely trapped; feeding then apparently ceases and the Aphid dies. The mortality rate is highest in first- and second-instar larvae which apparently cannot free themselves by pulling their legs off. There is also a high mortality during ecdysis when the impaled insects try unsuccessfully to emerge from the old cuticle. Richardson (1943) could find no evidence of the transfer of toxin to impaled insects and observations in the present work support this view.

The following experiments illustrate the various effects of the hooks on the Aphids.

Effect on Reproduction, Size and Longevity.*Reproductive rate on french beans and broad beans.*

As the upper leaf surfaces of french beans are almost devoid of hooks, it was possible to compare the reproductive rate of *A. craccivora* on french beans, without the inhibiting effects of the hooks, with that on broad beans which is a more suitable host and liable to heavy infestations in the field. Aphids were confined in cages to the upper leaf surfaces of both plants and the larvae were counted and removed every second day for 14 days. Average daily temperatures were 50°-68°F. The total numbers of larvae produced in 14 days are shown in Table I.



- Fig. 3. Epidermal hook penetrating the intersegmental membrane of an Aphid's leg between the tarsus and pretarsus with the point inside the tarsal claw.
Fig. 4. Hook embedded in the tarsus.
Fig. 5. Point of hook penetrating the tibia. The point can be seen inside the segment.
Fig. 6. Hook penetrating the intersegmental membrane between the tibia and tarsus.

There was no significant difference in the numbers of larvae produced on the two plants ($P = .01$). French beans are therefore physiologically suitable as a host plant of *A. craccivora* within the limits necessary for the following experiments.

TABLE I.

Total numbers of larvae produced by single apterae on the upper surface of french bean and broad bean leaves in 14 days.

	French beans	Broad beans
	29	27
	30	30
	51	37
	52	66
	53	68
Average ..	43	45.6
Av. no. larvae/day	3.1	3.3

Effect of hooks on reproductive rate and longevity.

Aphids placed on the lower surfaces of french-bean leaves were observed daily and their longevity and reproductive rate determined. The results are given in Table II with the data from the upper leaf surfaces from Table I.

TABLE II.

Longevity and reproductive rate of Aphids on upper and lower leaf surfaces of french beans.

Part of plant	No. of Aphids	Longevity (days)		Reproductive rate (larvae/day)	
		Average	Range	Average	Range
Upper leaf surfaces ..	5	14+*	—	3.1	2.1-3.8
Lower leaf surfaces ..	30	5.9	2-13	1.4	0.0-2.2

* After 14 days the Aphids on the upper leaf surfaces were still healthy.

The figures for the reproductive rate were obtained by dividing the total number of larvae produced each day by the number of surviving adult Aphids.

The epidermal hooks thus cause a considerable reduction in the longevity and reproductive rate of the Aphids. Reproduction on the hairy surfaces was normal for the first day or two until the effect of the hooks became apparent, then it decreased and very few larvae were produced after the sixth day.

TABLE III.

Length of Aphids matured on densely and sparsely hairy surfaces.

	Densely hairy surfaces	Sparsely hairy surfaces
Mean length of Aphids ..	1.20 mm.	1.43 mm.
Mean length (micrometer units)	29	34.5
s ²	9.6	6.3
n	47	41

Reduced size of Aphids.

Random samples of Aphids matured on densely hairy surfaces (3,000/sq. cm.) and sparsely hairy surfaces (1,000/sq. cm.) were taken and their length measured from the tip of the vertex to the posterior margin of the subgenital plate. The results are given in Table III.

The Aphids from the dense hair surfaces are smaller and the difference is significant ($P = .01$).

Size of Aphid and number of injuries.

Aphids with the greatest number of leg segments missing show the greatest reduction in size. The number of legs per Aphid with tarsus missing in the previous experiment was recorded. The results are given in Table IV.

TABLE IV.

Relationship between number of leg injuries per Aphid and Aphid size.

Length of Aphids	No. of leg injuries					Total injuries	Injuries per Aphid
	0	1	2	3	4		
	Nos. of Aphids						
0.9-1.1 mm.	2	1	3	1	6	34	2.6
1.1-1.3 mm.	0	4	10	0	5	44	2.3
1.3-1.5 mm.	4	4	4	2	1	22	1.5

There is a greater average number of injuries per Aphid with decreasing Aphid size (Table IV), but there is a large variation within each size group. Aphids may escape without losing part of a limb; but their feeding is interrupted, and this may explain why some dwarfs have no parts of their legs missing. On the other hand some of the larger Aphids have three and four injuries. These may have been received at a time (e.g., at the end of development) or in a way which had little effect on subsequent growth.

Relationship between Aphid size and reproductive rate.

Alpatov (1932) showed that dwarfed *Drosophila* from underfed larvae produced fewer eggs than flies of normal size. It is therefore possible that when Aphid growth has been inhibited by different hair-densities their fertility is reduced.

TABLE V.

Reproductive capacity of Aphids of different sizes.

Length of adults in mm.	Number of larvae produced	
	First 4 days	Subsequent 8 days
1.13	7	19
1.22	6	23
1.26	7	19
1.35	6	25
1.44	23	26
1.61	21	40
1.65	22	31
1.74	26	43

Young apterae of about the same age taken from french bean leaves (four each from densely and sparsely hairy surfaces), were placed on young cowpea plants and their reproductive rates recorded over 12 days. In the first four days the reproductive rates were so different for the two groups that a residual effect of the dense hairs, such as incomplete recovery from wounds, or previous starvation, is suspected. Only the numbers of larvae produced during the subsequent eight days have therefore been used in the regression.

Seventy-seven per cent. of the total sum of squares (554.1) was accounted for by regression which is significant at $P = .01$. Thus it appears that the reproductive capacity of individual Aphids is related to their size.

Effect of Hair Density on Larval Mortality.

Different parts of the plant.

The extent to which Aphids are affected depends upon the hair-density, which varies on different parts of the plant. The larval mortality and hair-density were compared on different parts of the variety Brown Beauty.

Large numbers of larvae were allowed to develop to maturity on the under-surfaces of the leaves; 20 to 50 larvae were used for each replicate. The variance was calculated between the different replicates within each group from the

formula variance = $\sqrt{\frac{1}{N-1} \sum (m-x)^2}$ $\times 100\%$, where x is the percentage mortality for each replicate, m the mean percentage mortality and N the number of replicates.

TABLE VI.

Larval mortality on different parts of french bean plant.

Part of plant	Density of hairs/sq. cm.	Total no. of larvae	% Mortality	Variance %	No. of replicates (N)
Growing shoot	30,000+	346	73	14.8	10
Mature leaflets	3,400	614	47	40.0	16
Primary leaves A* ..	3,200	206	44	19.1	9
" " B*	1,000	332	14	53.6	5
Petioles	1,000	150	13	38.4	5

* See p. 780.

The larval mortality is positively related to the density of the hairs. The mortality is highest on the growing shoots and least on the petiole and primary leaves B.

Different varieties.

Steinmetz and Arny (1932) describe some varieties of french bean as being more hairy than others. Hair-density counts were therefore made on nine varieties of beans grown in New South Wales.

The varieties fell into two distinct groups: dwarf varieties (Brown Beauty, Canadian Wonder, Tweed Wonder, Hawkesbury Wonder, and Burpee's Stringless) with 3-4,000 hairs/sq. cm., and runner varieties (Staley's Surprise, Scotia, Epicure and Scarlet Runner) with 700-1,800 hairs/sq. cm. Within these groups it was not possible to pick out more or less densely hairy varieties.

The larval mortality on two sparsely hairy varieties was compared with one densely hairy variety.

The same order of larval mortality is thus recorded on the varieties Scotia and Epicure as on the sparsely hairy primary leaves B of Brown Beauty (*viz.*, 14%) and these values are much lower than on the hairy leaves of the latter variety.

TABLE VII.

Larval mortality on different varieties of french beans.

Variety	Hair density /sq. cm.	No. of larvae	% Larval mortality	% Variance	No. of replicates (N)
Brown Beauty ..	3,400	614	47	47	16
Scotia	1,000	590	17	27	21
Epicure	1,000	212	17	47	9

Effect of hairs on length of larval development.

Development is retarded by the epidermal hairs. Numbers of larvae were deposited on leaf surfaces of varying hair-density and the period taken for all the survivors to reach maturity was recorded. The results are given in Table VIII.

TABLE VIII.

Length of larval development on densely and sparsely hairy surfaces.

	Cumulative percentages of total Aphids becoming adult after			Density of hairs/sq. cm.	Total number of larvae
	8 days	10 days	15 days		
Brown Beauty leaflets	10%	50%	100%	3,400	92
" " leaves B	50%	100%		1,000	151
Scotia leaflets	25%	100%		1,000	654

On the surfaces with sparse hairs the larvae matured fairly uniformly whereas among the dense hairs development was lengthened and more variable.

Discussion.

In the absence of the hooked epidermal hairs, *Aphis craccivora* can survive and reproduce on french beans as successfully as on broad beans and cowpeas which are liable to heavy infestations in the field. Thus were it not for the epidermal hooks, french beans would probably be more liable to heavy aphid infestations than is the case. Sometimes large colonies are found on the petioles and fruit, but mostly the Aphids settle on the undersurfaces of the leaves where, on hairy varieties, the hooks are most dense and the resulting colonies do not become well established. Aphids will not normally remain feeding on the upper surface of a leaf unless this is directed downwards.

The effect of the hooks increases with their density; they are most dense and therefore most injurious to the Aphids on the young growing shoots which would normally be most favoured.

Of the different varieties tested, the dwarf beans were all found to have dense hairs and would thus be expected to be fairly resistant to Aphid infestation. On

the basis of the above experiments, the runner beans should be more liable to infestations. No field data was available on the relative susceptibility of different varieties.

Dwarf beans grown in the coastal regions of New South Wales are often damaged by large numbers of migrant Aphids soon after their emergence, and although the epidermal hooks inhibit the formation of large colonies, they may be partly responsible for the extent of the damage by retaining the migrants on the plants. If this is so, the hooks may also be instrumental in reducing the spread of virus diseases, by reducing the number of Aphids taking off from infected plants.

The beneficial effects of the hooks, however, are offset to some extent by other factors, such as reducing the efficiency of predators. Schneider (1944) thought that the effect of the hooks on the predators was more important than their effect on the Aphids and it seems probable therefore that he made his observations on sparsely hairy varieties where the Aphid mortality is low (see above). On densely hairy surfaces populations of Aphids and predators are both decreased but the high mortality of the Aphids prevents them from establishing large colonies; on sparsely hairy surfaces the predators may be more liable than the Aphids to contact the sparsely scattered hairs due to their greater activity, and their efficiency may thus be reduced while the Aphids are able to become established; and therefore on sparsely hairy varieties of beans the hooks may become a liability to the plant.

Summary.

The small, hooked epidermal hairs on french beans (*Phaseolus vulgaris*) may have a profoundly detrimental effect on colonies of *Aphis craccivora*. The hairs are present on the petioles, stems and undersurfaces of the leaves but are absent on the uppersurfaces of the leaves. They are most dense and therefore most injurious to the Aphids on the growing shoots. The Aphids' legs become impaled on the hooks and the results of the subsequent bleeding, starvation and exhaustion are decreased longevity and reproductive rate, high larval mortality, increased time of larval development and decreased size; this last factor is associated with a reduced fecundity. On some varieties of beans the hairs are less dense than on others and on these varieties the action of the hairs in inhibiting predators may be more important than their effect on the Aphids.

Acknowledgements.

I am indebted to Dr. A. R. Woodhill under whose supervision the work was carried out, to Professor P. D. F. Murray for taking the photomicrographs, and to Dr. C. G. Johnson for much helpful criticism of the paper during its preparation.

References.

- ALPATOV, W. W. (1932). Egg production in *Drosophila melanogaster* and some factors which influence it.—J. exp. Zool., **63**, pp. 81–111.
- V. BOGDANDY, ST. (1927). Ausrottung von Bettwanzen mit Bohnenblättern.—Naturwissenschaften, **15**, p. 474.
- DE FLUITER, H. J. & ANKERSMIT, G. W. (1948). Gegevens betreffende de aantasting van bonen (*Phaseolus vulgaris* L.) door de zwarte bonenluis (*Aphis* (*Doralis*) *fabae* Scop.)—Tijdschr. PlZiekt., **54**, pp. 1–13.
- HELY, P. C. (1945). Fruit flies (*Strumeta tryoni*) trapped by bean leaves.—Agric. Gaz. N.S.W., **56**, pp. 22–23.

- HABERLANDT, G. (1914). Physiological Plant Anatomy.—794 pp. London, Macmillan.
- McKINNEY, K. B. (1938). Physical characteristics on the foliage of beans and tomatoes that tend to control some small insect pests.—J. econ. Ent., **31**, pp. 630-631.
- MILLER, L. W. (1947). Populations of *Thrips tabaci* Lind. on bean varieties.—J. Aust. Inst. agric. Sci., **13**, pp. 141-142.
- POOS, F. W. & SMITH, F. F. (1931). A comparison of oviposition and nymphal development of *Empoasca fabae* (Harris) on different host plants.—J. econ. Ent., **24**, pp. 361-371.
- RICHARDSON, H. H. (1943). The action of bean leaves against the Bedbug.—J. econ. Ent., **36**, pp. 543-545.
- SCHNEIDER, F. (1944). Eine Ursache der raschen Blattlausvermehrung an Bohnen.—ForschErgebn. Gartenb., **1944**, no. 5, repr. 4 pp.
- STEINMETZ, F. H. & ARNY, A. C. (1932). A classification of the varieties of field beans, *Phaseolus vulgaris*.—J. agric. Res., **45**, pp. 1-50.
- TROUVELOT, B. & THENARD, J. (1931). Remarques sur les éléments des végétaux contribuant à limiter ou à empêcher la pullulation du *Leptinotarsa decemlineata* Say sur de nombreuses espèces ou races végétales.—Rev. Path. vég., **18**, pp. 277-285.
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REPORT ON ENCYRTIDAE ASSOCIATED WITH MEALYBUGS ON CACAO IN TRINIDAD, AND ON SOME OTHER SPECIES RELATED THERETO.

By G. J. KERRICH.

Commonwealth Institute of Entomology.

The primary purpose of the present paper is to give taxonomic treatment to a complex of parasites associated with mealybugs on cacao in Trinidad, reared by Professor T. W. Kirkpatrick, whose study of the biology of these insects has been prepared for publication. New species have been described, and species not new have been treated in comparable detail. Fresh taxonomic or other data have been given for some related species, which seemed to fit in naturally with this study. The inclusion of an aberrant Thysanid species of biological importance compelled a comparative study of characters that have been used within this group, in order that a generic placing of the species might be given and justified. Although this problem has not been solved to the author's satisfaction, it is hoped that the presentation of data on the species relationships may point the way to a further advance in knowledge. Thysanid parasites of TACHINIDAE attacking moth larvae in sugar-cane were considered in this connection, and were found to be separable into three closely related species.

Most of the material treated, including the types of all new species, is in the British Museum (Natural History).

Family ENCYRTIDAE.

Genus *Leptomastix* Förster.

Leptomastix Förster, 1856, Hymenopterologische Studien, **2**, p. 34.

Leptomastix Compere, 1947, Univ. Calif. Publ. Ent., **8**, pp. 17-18.

Leptomastix dactylopii Howard.

Leptomastix dactylopii Howard, 1885, Bull. U.S. Bur. Ent., no. 5 pp. 23-24.

Leptomastix dactylopii Dozier, 1927, J. Dep. Agric. P.R., **10**, (3-4) pp. 267-269.

Leptomastix dactylopii Compere, 1939, Univ. Calif. Publ. Ent., **4**, pp. 57-58.

Material. California, Riverside, 2 ♀♀ 1935, from ancestors from Brazil, propagated on *Pseudococcus citri* (Risso) (see Compere, 1939b): Brazil, Campinas, 1 ♀ ex *Pseudococcus inamabilis* Hambleton, 26.xii.1935, E. Hambleton; det. H. Compere: Trinidad, St. Augustine, 2 ♂♂ numerous ♀♀ ex *Pseudococcus citri* (Risso) on cacao, i-v.1949, T. W. Kirkpatrick: Bermuda, numerous ♂♂ ♀♀ ex *Pseudococcus citri* (Risso) on potato, 10.viii.1950, F. D. Bennett.

This is the species *dactylopii* Howard as interpreted by Compere (1939b). That author gave a colour redescription in order to distinguish it from a new Brazilian species *bahiensis* described in the same paper; the structural characters being illustrated in the figure (his fig. 1), or implied by comparison with the description of *bahiensis*. He pointed out that there were discrepancies from the original description. I did not find satisfactory agreement of the Trinidad material with description of Dozier (1927). Dr. Compere, however, very kindly sent me specimens from the Riverside stock, and I agree that the Trinidad species is the same and, of course, that it conforms with Compere's redescription.

L. dactylopii is capable of reproducing parthenogenetically (Compere, *in litt.*) and, in fact, the Trinidad series as submitted is predominantly female. The series from Bermuda, however, is composed of both sexes in approximately equal numbers.

Since a third tropical American species is to be described, it seems desirable to give comparative short descriptions of all three. The description of *L. dactylopii* is given immediately below.

Fronto-vertex weakly reticulate: ocelli in an obtuse triangle, sides to base $9\frac{1}{2}$: 12 measured from mid points of ocelli; the lateral ocelli about one and a third times their own diameters from the eyes, and less than twice in female, and once in male, their own diameters from the occipital margin: eyes not appreciably hairy (magnification 100): scrobes joined above by a distinct transverse furrow in female, weaker in male. Mesoscutum with sides weakly explanate: scutellum and axillae alutaceous.

Vertex, metathorax and propodeum chrome yellow: mesoscutum and scutellum golden-yellow to golden-brown, seldom very much infuscate: scape beneath, lower face, cheeks, mesopleura and coxae all a bright yellow.

Scape above and radical segment, pedicellus above, funicle and club ferrugineous; the pedicellus paler at apex and usually beneath.

Female—scape five times as long as wide: pedicellus not quite half length of first funicle segment: sixth funicle segment twice as long as wide. Antennae inserted much nearer eyes than oral margin.

Male—scape just over four times as long as wide.

Leptomastix bahiensis Compere.

The following description has been made from two of the paratypes, is partly adapted from that of Compere (1939b), and is intended to compare with the descriptions of the other two species concerned.

Fronto-vertex strongly reticulate: ocelli (see Compere, 1939b) in an acute triangle: eyes strongly hairy: scrobes not distinctly joined above. Mesoscutum with sides strongly explanate: scutellum and axillae alutaceous.

Dominantly testaceous, with fronto-vertex more yellowish: face from top of scrobes, and cheeks much paler.

Colour of scape and pedicellus (see Compere, 1939b): funicle ferrugineous, club white. Scape just over three times as long as wide: pedicellus little shorter than first funicle segment: sixth funicle segment slightly longer than wide (4:3). Antennae inserted about mid way between eyes and oral margin.

Fore wings (see Compere, 1939b): when this species is compared with that described below, the basal infuscation appears as a more or less definite secondary cross band, which broadens posteriorly.

Male unknown.

Leptomastix dispar, sp.n.

Fronto-vertex still more strongly reticulate than in *bahiensis* Comp.: ocelli in an obtuse triangle, sides to base 10: 14 measured from mid points of ocelli; the lateral ocelli a little less than their own diameters from eyes in female but a little more in male, and less than twice in female and once in male their own diameters from occipital margin: eyes strongly hairy: scrobes not very distinctly joined above. Mesoscutum with sides a little less strongly explanate than in *bahiensis* Comp.: scutellum and axillae distinctly, finely reticulate.

Female—fronto-vertex, thorax above and propodeum a rich testaceous, the thorax often weakly infuscate and with weak metallic reflections: face from top of scrobes, cheeks, pleura, and fore and hind coxae a paler testaceous: mid coxae largely infuscate: hind tibiae in greater part infuscate: mid tibiae infuscate at

sides, except at base and apex: gaster extensively infusate above, but usually conspicuously pale at apex.

Scape broadly pale testaceous in middle, with dorsal and ventral sides and radical segment ferrugineous: pedicellus ferrugineous, pale at apex and beneath: funicle with first five segments ferrugineous; with sixth, and sometimes fifth in part, like the club, white. Scape (fig. 1) four times as long as wide: pedicellus

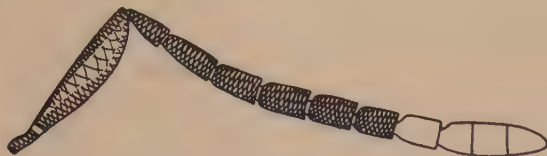


Fig. 1.—*Leptomastix dispar*, sp.n. Right antenna of ♀ in dextro-lateral view.

three-fourths length of first funicle segment: sixth funicle segment distinctly longer than wide (8:5). Antennae inserted a little nearer oral margin than eye (when there is no distortion).

Fore wings with pattern essentially similar to that of *bahiensis* Comp.; but crossbands decidedly, usually very much, darker; the main crossband has a distally directed paler extension to the hind margin; the wing is weakly infusate at base and apex; and the speculum is not very conspicuous.

Male—thorax and abdomen above brownish-infusate, with metallic reflections that are weak yet stronger than in female, the sclerites mostly pale marginally: scape and pedicellus above similar: vertical region and flagellum similar but more weakly so: upper face, mesopleura, and mid and hind coxae usually dull testaceous: scape beneath, lower face and cheeks, pronotum at sides, and fore coxae usually dirty white. Legs pale, with tibiae not infusate. The paler parts are not nearly so brightly coloured as those of *dactylopii* How. Scape less than four times as long as wide.

Material. Trinidad, Imperial College of Tropical Agriculture, 13 ♂♂, 24 ♀♀ (including holotype) *ex Ferrisiana virgata* (Ckll.) on cacao, xi.1949, T. W. Kirkpatrick.

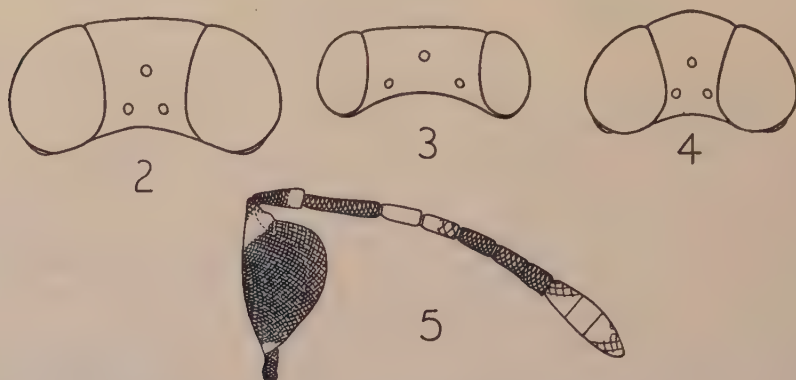
The trivial name was suggested by Professor Kirkpatrick's comment that this species has an unusual degree of sexual dimorphism for a *Leptomastix*.

KEY TO TROPICAL AMERICAN SPECIES OF *Leptomastix*.

1. Fronto-vertex weakly reticulate: eyes not appreciably hairy ($\times 100$): body dominantly various shades of bright yellow; female funicle and club ferrugineous: fore-wing with no concentrated infuscation in either sex *dactylopii* How.
- Fronto-vertex quite strongly reticulate: eyes strongly hairy: body dominantly testaceous, often paler below and with infuscation above: antennal club of female white: forewing of female with diagonal infusate cross-band traversing stigma and a weaker band or area nearer base 2
2. Ocelli in an acute triangle: scutellum and axillae alutaceous: female scape just over 3 times as long as broad: female with funicle wholly ferrugineous: male unknown *bahiensis* Comp.
- Ocelli in an obtuse triangle: scutellum and axillae distinctly, finely reticulate: female scape 4 times as long as broad: female with sixth funicle segment, like the club, white *dispar*, sp.n.

Genus **Apoanagyrus** Compere 1947.

This genus was erected for an apparently very variable new species *californicus*, of which certain series were designated as typical, and a second species which was not validated owing to the inadequacy of the material. In that second species, the female antenna has the first funicle segment scarcely longer than the second, and sub-equal in length to the pedicel. The species described below agrees with *californicus* Comp. in having the female first funicle segment much longer than either the pedicel or the second funicle, but differs from it in having the female flagellum much more slender yet the scape much more strongly expanded below (fig. 5).



Figs. 2-4.—Head, viewed from above, of: (2) *Apoanagyrus californicus* Comp. ♀; (3) *A. californicus* ♂; (4) *A. trinidadensis*, sp.n. ♀.

Fig. 5.—*A. trinidadensis*, sp.n. Right antenna of ♀ in dextro-lateral view.

***Apoanagyrus trinidadensis*, sp.n.**

Female—sculpture and vestiture as described for *californicus* Comp., or if anything a little finer: in both species the reticulations near median ocellus (Compere, 1947, fig. 7) are just clearly distinguishable $\times 25$, and the mesoscutum is beset with rather superficial punctures on an alutaceous background.

Head from above about one and a half times as broad as long (fig. 4): fronto-vertex relatively narrower than in *californicus* (across narrowest point 19:34): lateral ocelli much less than their own diameters from eyes [in *californicus* about their own diameters from eyes].

Antennae (fig. 5) with scape much more strongly expanded below than in *californicus* Comp., but with flagellum much more slender. Scape about one and a half times as long as wide: funicle with first segment about five times and fourth more than twice as long as wide, with sixth segment distinctly shorter than fifth and distinctly expanded to base of club.

Colour of head, body, scape and pedicellus much as in *californicus* (Compere, 1947, p. 19); but the head more, as it were, enamelled, and the scape distinctly metallic shining and with the whitish mark smaller. Flagellum blackish to brownish, with club and with second and third funicle segments distinctly so towards apex. Legs stramineous, with mid and hind coxae except at apex, and hind femora in greater part, metallic brownish to greenish black; with mid femora darkened above near apex. Both species have the palpi whitish, the first large abdominal tergite greenish metallic, and a crescentic pale mark or a trace of such before the tegulae.

Male—with scape rather cigar-shaped in broadest view, not more expanded below than in *californicus* Comp.: with scale-like hairs beneath sixth funicle and first club segments much stronger than in that species.

In both *californicus* and *trinidadensis* the flagellum is pale brown and the scape ferrugineous, pale at base; but in *californicus* this pale part is stramineous, and occupies about two-fifths of the scape, whereas in *trinidadensis* it is much smaller and less conspicuous. Leg colour much as described for the female, but tarsi and hind tibiae more a pale, dull brown.

Material. Trinidad, Imperial College of Tropical Agriculture, 5 ♀♀ (including holotype), 1 ♂ *ex Ferrisiana virgata* (Ckll.) on cacao, viii.1949, T. W. Kirkpatrick.

KEY TO DESCRIBED SPECIES OF *Apoanagyrus*.

Female scape about $2\frac{1}{2}$ times as long as broad: head, notably fronto-vertex, broader in each sex respectively (figs. 2, 3): fore coxae almost always dark *californicus* Compere
 Female scape about $1\frac{1}{2}$ times as long as broad (fig. 5): head, notably fronto-vertex, narrower in each sex respectively (fig. 4): fore coxae stramineous *trinidadensis*, sp.n.

Genus *Neodiscodes* Compere 1931.

A very distinct new species of this genus has been reared by Professor Kirkpatrick, and is described below. This work has led to a review of the known material of the genus.

Compere (1931) based the genus on a single new species, *martinii* Comp., from Eritrea. In 1939, he attributed two further series to the same species, one reared from *Pseudococcus* sp. at Kilifi, Kenya, the other from a mealybug on Kei apple at the Scott Agricultural Laboratory, Nairobi, Kenya. Two specimens of the former and one of the latter were deposited in the British Museum (Natural History). A re-examination of these specimens indicates that the series from Kilifi is more or less typical *martinii*, in sculpture of the fronto-vertex and in other respects; but the specimen associated with Kei apple has the sculpture of the fronto-vertex different, as indicated by Compere (1939a), and has other slight differences also. In the light of the further data now available, it seems that this latter may be a distinct species: in the present paper it is not validated as such, but it is diagnosed in order to draw attention to its existence. The rearings from mealybugs on cacao in the Gold Coast are not of this form, but are all typical *martinii*.

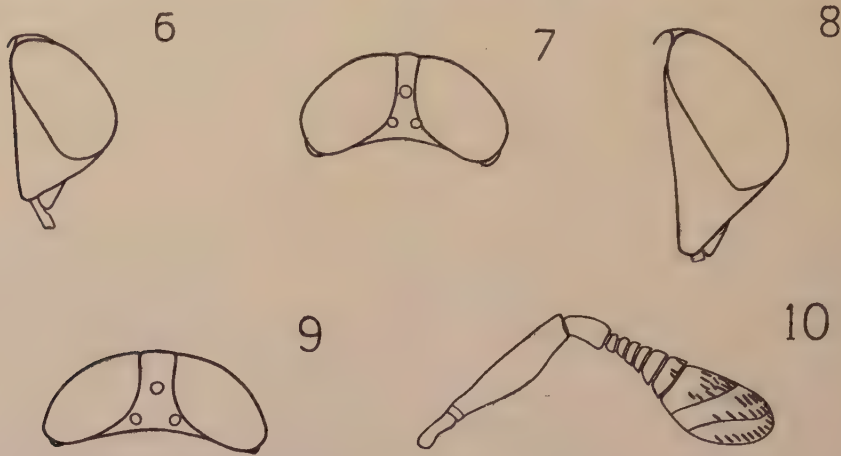
Finally, a new species reared by Dr. R. H. Le Pelley from *Pseudococcus lilacinus* (Ckll.) in Ceylon is described, and a key is given for the separation of the four forms now known.

Neodiscodes kirkpatricki, sp.n.

Head in side view (fig. 6) even longer than in *martinii* Comp., with malar space much shorter than short diameter of eye: fronto-vertex so narrow that the median ocellus seems almost to merge into the eye, its distance from eye being decidedly less than half its diameter (fig. 7); shagreened, with a row of fine piliferous punctures along each inner orbit and with scattered punctures, coarser but shallow, between, not in any part reticulate-punctate: scrobes and interscrobal prominence shining: eyes weakly hairy, the hairs scarcely discernible $\times 20$. Mandibles with lowest tooth shorter than in *martinii* (Compere, 1931, fig. 3b), nearer in length to the uppermost than to the middle. Antennae with scape only slightly dilated below, that of female almost five times length of its greatest breadth (fig. 10). Thorax and propodeum as described for the genus (Compere, 1931, p. 273), but with dorsum more deeply punctate and mesoscutum

more strongly convex than in *martinii* Comp.: propodeum coarsely white-hairy round spiracle.

Head metallic green to bluish and bronzy, with the shining facial area more brassy. Antennae blackish-brown, with scape and pedicellus narrowly pale at



Figs. 6-10.—(6) *Neodiscodes kirkpatricki* sp.n., head of ♀ in dextro-lateral view; (7) the same, viewed from above; (8) *N. lepelleyi*, sp.n., head of ♀ in dextro-lateral view; (9) *N. martinii* Comp., head of ♀ from above; (10) *N. kirkpatricki*, sp.n., antenna of ♀ in dextro-lateral view.

apex. Thorax, propodeum and gaster rather bright metallic green to bronzy, the green most conspicuous on mesoscutum and sides of propodeum, and the scutellum mostly bronzy, the mesoscutum with some infusion of bluish, and the gaster rather paler beneath. Legs blackish-brown, the fore and mid femora and tibiae paler in part, especially at apex: spur of mid tibiae whitish: tarsal coloration as described for *martinii* Comp. (see below).

In male the mesoscutum is bronzy, not or scarcely at all green, and the shining facial area is green, not conspicuously brassy.

Material. Trinidad, St. Augustine, 1 ♀, 1 ♂, 1.v.1949, *ex Pseudococcus* sp. on cacao; Imperial College of Tropical Agriculture, 14 ♀♀ (including holotype), 2 ♂♂, 1950, *ex Pseudococcus* sp. near *brevipes* (Ckll.) on cacao, T. W. Kirkpatrick.

***Neodiscodes martinii* Compere.**

Head in side view rather long, with malar space shorter than short diameter of eye: fronto-vertex coarsely reticulate-punctate, shagreened behind lateral ocelli; with median ocellus separated from eyes by about two-thirds its own diameter, scrobes and inter-scrobal prominence shagreened, not shining: eyes strongly hairy, distinctly so $\times 20$. Antennae with scape strongly dilated below, about $2\frac{1}{2}$ times length of its greatest breadth. See Compere (1931, p. 273) for figure of mandible (fig. 3b) and description of thorax and propodeum.

Head metallic blue to blue-green, sometimes in part virescent or bronzy. Antennae blackish brown, with scape and pedicellus paler at apex. Dorsum of thorax and propodeum with dull grey-green and bronzy metallic sheen: lateral and ventral parts and gaster brownish, with slight metallic sheen. Legs blackish-brown, the mid and hind femora and tibiae paler in part, least so the hind tibiae: fore tarsi paler: mid and hind tarsi, except apical segments, stramineous, brownish below and by segmental apices. Male not notably different.

Material. Eritrea, Nefasit, 1 ♀ (paratype), *ex Pseudococcus citri* (Risso) on wild olive, iv.1930, H. Compere: Kenya, Kilifi, 1 ♀ 1 ♂, *ex Pseudococcus* sp., 13.viii.1937, A. R. Melville: Gold Coast, Tafo, *ex Pseudococcus njalensis* Laing on cacao, 1 ♀ 1 ♂, xi.1945, 8 ♀ ♀ 3 ♂♂ 1947, 8 ♀ ♀ 2 ♂♂ 1949, A. H. Strickland.

Neodiscodes sp.

In all respects as described for *martinii* Comp., except as follows: fronto-vertex shagreened, with scattered punctures which become coarser and closer just above scrobal area, and also with a row of fine piliferous punctures along each orbit; with median ocellus separated by about its own diameter from eye margin: eyes weakly hairy, the hairs just clearly discernible $\times 40$: antennal scape perhaps a little less strongly dilated below: dorsum of thorax more deeply punctate. Paler parts of femora and tibiae more conspicuously pale.

Material. Kenya, Nairobi, Scott Agricultural Laboratory, 1 ♀ *ex Pseudococcus* sp. on Kei apple, 6.iii.1937, A. R. Melville.

Neodiscodes lepelleyi, sp.n.

Head, in side view (fig. 8) shorter than in the other species, with malar space slightly longer than short diameter of eye: fronto-vertex shagreened, with scattered punctures which become coarser and closer just above scrobal area; with median ocellus clearly separated by about half its own diameter from eye margin: scrobes and inter-scrobal prominence shagreened, not shining: eyes weakly hairy, the hairs just clearly discernible $\times 40$. Mandibles with lowest tooth not much shorter than middle. Antennae with scape strongly dilated below, about $2\frac{1}{2}$ times length of its greatest breadth. Thorax and propodeum as described for the genus (Compere, 1931, p. 273), but the dorsum rather more deeply punctate than in *martinii* Comp.

Head metallic green and bronzy. Antennae blackish, with no segment distinctly paler at apex. Thorax, propodeum and gaster with dull grey-green and bronzy metallic sheen, the other parts not notably paler than the thoracic dorsum. Leg colour as described for *martinii* Comp.

Material. Ceylon, Peradeniya, 2 ♀ ♀ 1 ♂ 11.vii.1937, *ex Pseudococcus lilacinus* (Ckll.), 1 ♀ 5.viii.1937, supposedly *ex Scymnus* sp. (Coccinellidae) (mixed with a long series of *Homalotylus* sp.), R. H. Le Pelley.

KEY TO SPECIES OF *Neodiscodes*.

1. Fronto-vertex so narrow that the median ocellus seems almost to merge into the eye, its distance from eye being decidedly less than half its diameter (fig. 7): scrobes and inter-scrobal prominence shining: scape only slightly dilated below, almost 5 times length of its greatest breadth: propodeum coarsely white-hairy round spiracle: mid tibial spur whitish: neotropical species *kirkpatricki*, **sp.n.**
 Fronto-vertex less narrow, with median ocellus clearly separated from eye by about half its own diameter or more: scrobes and inter-scrobal prominence shagreened, not shining: scape strongly dilated below, about $2\frac{1}{2}$ times length of its greatest breadth (Compere 1931, fig. 3a, e): propodeum not coarsely white-hairy round spiracle: mid tibial spur brownish: tropical Old World species 2
2. Fronto-vertex coarsely reticulate, the punctures deep, shagreened behind lateral ocelli: eyes strongly hairy, distinctly so $\times 20$ *martinii* Comp.
 Fronto-vertex shagreened, the punctures shallow and mostly well-separated: eyes weakly hairy, the hairs just clearly discernible $\times 40$ 3
3. Agreeing with the above two species in having the head, in side view, longer, the malar space shorter than short diameter of eye, and in having the

scape and pedicellus distinctly paler at apex: agreeing also with *martinii* Comp. in having the thoracic dorsum notably darker than the pleural and ventral parts and the gaster: median ocellus separated by about its own diameter from eye margin: Kenya sp.
 Head, in side view, shorter, the malar space slightly longer than short diameter of eye: antennae with no segment distinctly paler at apex: other parts of body not notably paler than thoracic dorsum: median ocellus separated by about half its own diameter from eye margin: Ceylon
lepelleyi, sp.n.

Genus **Aenasius** Walker 1846.

This genus has been well revised by Compere (1937), who gave a key for its separation from its nearest relatives.

Aenasius theobromae, sp.n.

This species runs down in the key of Compere (1937) with *cariocus* Comp., from which it is quite distinct.

Head from above not nearly twice as wide as long (28:17) [in *cariocus* about twice as wide, 34:16]: fronto-vertex at narrowest with six rows of punctures: facial impression about half height of head, weakly gibbous between the toruli; and eyes, to either side of it, more sharply diverging than in *cariocus* Comp.: ocelli in a slightly obtuse triangle. Antennae with scape about $3\frac{1}{2}$ times length of its greatest breadth; with club, in dry specimens, appearing relatively broader than that of *cariocus*. Mesoscutum and scutellum much as in *cariocus*, but with a less shining and more strongly alutaceous background. Wing venation similar to that of *cariocus*.

Fronto-vertex brassy green, with the ocellar area darker: facial impression marginally, and cheeks bronzy. Antennae with scape and funicle yellow, the scape darkened below in basal half; with pedicellus and club pale blackish-brown, the pedicellus with metallic reflections above. Thorax and abdomen blackish, with metallic reflections, on mesoscutum and scutellum green and on pleura and abdomen bronzy. Legs blackish-brown, with the following dirty yellow-brown, marginally dusky: fore and hind femora at apex, mid femora in greater part, and all tibiae; with all tarsi pale yellow-brown, and with spurs of fore and mid tibiae dark.

Male—in this species and in *cariocus* Comp. the antennae are of the type of those of *hyettus* Walk. (Compere 1937, fig. 1), but the club is relatively not so broad. The head and body colour are dull bronzy, with the facial impression greenish to bluish. The mesoscutum and scutellum have fine reticulate sculpture, and superficial punctures are discernible on the mesoscutum clearly in *cariocus* Comp. but not in *theobromae* sp.n.

Material. Trinidad, Maracas, 2 ♀♀ (including holotype), 1 ♂ *ex Pseudococcus brevipes* (Ckll.) on cacao pod, x.1949, T. W. Kirkpatrick.

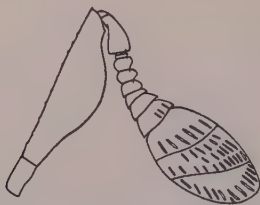


Fig. 11.—*Aenasius theobromae*, sp.n. Right antenna of ♀ in dextro-lateral view.

KEY FOR THE SEPARATION OF THE FEMALES OF *A. theobromae* AND *cariocus*.

Head about twice as wide as long (34:16): fronto-vertex at narrowest with 5 rows of punctures: facial impression about one-third height of head: antenna (Compere 1937, fig. 2): fronto-vertex at most with green reflections, purplish-blue, with ocellar area violaceous black: sixth funicle segment dark

cariocus Comp.

Head not nearly twice as wide as long (28:17): fronto-vertex at narrowest with 6 rows of punctures: facial impression about half height of head: antenna (fig. 11) with club relatively broad: fronto-vertex brassy green, with the ocellar area darker: sixth funicle segment pale *theobromae*, sp.n.

***Aenasius masii* Domen.**

Dr. G. Domenichini has kindly sent me on loan a specimen of his new Peruvian species. As he states (1952, p. 17), it runs in the key of Compere (1937) to *caeruleus* Brues, a species not known to me. It appears to me to be related to *advena* Comp. in the short head (Domenichini, fig. 4), with ocelli in an acute triangle, and the facial impressions strongly gibbous between the toruli; but the antennae differ in the proportions of the segments as well as in colour.

***Aenasius advena* Comp.**

This species was described (1937) from the Hawaiian Islands. It has now been received from Fiji, Naduruloulou, 2 ♀♀ 4 ♂♂ ii.1949, *ex* mealybug on *Albizia lebbek*, B. A. O'Connor.

Genus *Coccidoctonus* Crawford 1912.

This genus is here recorded, in one and the same neo-tropical species, as a secondary parasite of a mealybug, and as a parasite of a predatory midge and of a ladybird. The parasitism of Cecidomyiidae associated with mealybugs is supported with other records from Africa.

***Coccidoctonus trinidadensis* Crawford.**

Coccidoctonus trinidadensis Crawford, 1912.—Proc. U.S. nat. Mus., **43**, pp. 167–168.

Material. Trinidad, St. Augustine, 1 ♂ 14 ♀♀, *ex* *Leptomastix dactylopii* How. on *Pseudococcus citri* (Risso) on cacao, i–v.1949, T. W. Kirkpatrick: Imperial College of Tropical Agriculture, 2 ♂♂ 8 ♀♀, *ex* Cecidomyiid predator of eggs of *P. citri* on cacao, i.1950, T. W. Kirkpatrick: Barbados, 1 ♂ 2 ♀♀, “*ex* mealy bug and Coccinellid”, of which 1 ♀ labelled additionally “*ex* *Cryptolaemus* sp.”, 1935, R. W. E. Tucker.

I have examined these three series, and am satisfied that all belong to the same species, and agree with the original description. The specimens of the first-mentioned series are rather darker and more slender than the others. Professor Kirkpatrick is definite that this series was reared as a secondary parasite of the mealy-bug, but the second series as a parasite of the predatory midge. The type series was reared “from *Pulvinaria pyrifomis* on honey-suckle”, Port of Spain, Trinidad.

***Coccidoctonus* species.**

Material. Tanganyika, Bukoba, 1 ♂ 1 ♀, “*ex* mealy-bug Cecidomyiid”, vi.1935, A. H. Ritchie: Belgian Congo, Kivu, 1 ♂ 3 ♀♀, “*ex* midge pupa”, 5.vii. and 12.x.1937, A. R. Melville.

These series certainly differ specifically from *trinidadensis* Crawf., and probably also from each other. The host and locality records for the genus are thought to be of interest.

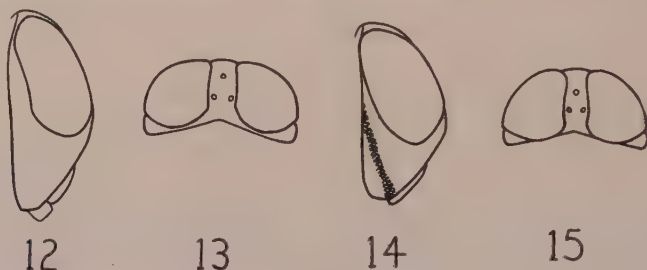
Genus **Achrysopophagus** Girault 1915.

Two species involved in this parasite complex are referred to the genus *Achrysopophagus* Girault. For their determination, I am much indebted to Dr. B. D. Burks of Washington. Provisionally, I had placed the first species as *seini* Dozier 1927, by reference to Dozier's paper, and the second as an undescribed species. I submitted specimens of the two species to Dr. Burks, together with my descriptive treatment of them, with the request that he determine them by comparison with the types. In reply, he wrote that the first species was *dactylopii* (Howard) and the second *seini* Dozier. Since Dozier's description and figures had not led me to a determination with which Burks could agree, I have figured the female antennae of the two species afresh.

Achrysopophagus dactylopii (Howard) ♀.

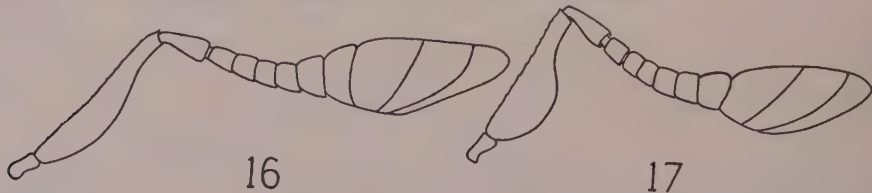
Chiloneurus dactylopii Howard, 1885, Bull. U.S. Bur. Ent., no. 5 p. 17.

Head from above (fig. 13) rather short and broad, about two and a half times as broad as its mid length; in side view (fig. 12) with malar space longer than short diameter of eye: eyes, as seen in figs. 12-13, not reaching occipital margin; closely hairy, the hairs discernible with difficulty $\times 40$. Surface of fronto-vertex about as in *Leptomastix dactylopii* How., and more finely sculptured than in *Achr. aegyptiacus* Merc. Antennae (fig. 16) with scape very little dilated below, about five times length of its greatest breadth. Rather conspicuous black hairs arise usually from near hind margin of pronotum, and always from axillae and tegulae. In dorsal aspect, ovipositor projecting beyond apex of gaster by about length of gaster behind first large tergite.



Figs. 12-15.—(12) *Achrysopophagus dactylopii* (How.), head of ♀ in dextro-lateral view; (13) the same, viewed from above; (14) *A. seinii* Doz., head of ♀ in dextro-lateral view; (15) the same, viewed from above.

Fore wings with hyaline area in lower half of infumate part extending from level of base of marginal to apex of radius and pterostigma.



Figs. 16-17.—Right antenna of ♀, in dextro-lateral view: (16) *Achrysopophagus dactylopii* How.; (17) *A. seinii* Doz.

Species of *Cheiloneurus* and related genera may be very variable in colour (cf. Compere, 1938, pp. 330-1). In the females of the present bred series, the following points are noteworthy: cheeks with silvery-blue metallic sheen, and with a weak, vertical dark stripe behind this: antennal scape whitish, with dark stripe along upper and lower margins and with dark mark at apex: pedicellus and first four or five funicle segments pale above, but all more or less dark-marked at sides: propodeum strongly blue-green and violet metallic coloured at sides: legs stramineous-white; with fore femora in about apical third, hind femora in about apical two-thirds, all tibiae mainly, and fore tarsi pale testaceous: all femora and tibiae have more or less conspicuous dark streaks, and the hind tibiae are conspicuously stramineous-white at base.

Material. Trinidad, St. Augustine, 27 ♀ ♀ hyperparasitic on *Pseudococcus citri* (Risso) on cacao, through *Leptomastix dactylopii* How. (see above), i-v.1949, T. W. Kirkpatrick.

Achrysopophagus seini Dozier ♀.

Achrysopophagus seini Dozier, 1927, J. Dep. Agric. P.R., 10 (3-4) pp. 269-270.

Head from above (fig. 15) longer and narrower than in *dactylopii* (How.); about two and a quarter times as broad as its mid length; in side view (fig. 14) with malar space shorter than short diameter of eye: eyes, as seen in figs. 14-15, over-reaching occipital margin; more sparsely hairy, the hairs darker, just clearly discernible $\times 60$. Sculpture of fronto-vertex about as fine as in *dactylopii* (How.). Antennae (fig. 17) with scape rather strongly dilated below in about basal half, less than four times length of its greatest breadth. Black hairs are usually developed on pronotum, rather densely on fore part and a few stronger ones near hind margin of mesoscutum, and always on axillae: they are very strong and conspicuous on tegulae. In dorsal aspect, ovipositor projecting beyond apex of gaster, by somewhat less than length of gaster behind first large tergite.

Fore wings with hyaline area in lower half of infumate part longer than in *dactylopii* (How.), extending well beyond level of radius and pterostigma.

Differs from *dactylopii* (How.) in colour in several respects: cheeks with pale green metallic sheen before a strong, vertical dark stripe: antennal scape pale testaceous, whitish near apex, darkened beyond and usually before the whitish part: propodeum more weakly metallic coloured at sides: [leg colour not notably different].

Material. Trinidad, 13 ♀ ♀, same data as for *dactylopii* (How.), T. W. Kirkpatrick.

The female of this species is best separated from *dactylopii* How. by the shape of the head, with the eyes over-reaching the occipital margin; by the antennal scape, which is rather strongly dilated below; and by the fore wings having a longer hyaline area in the lower half of the infumate part.

Males of *Achrysopophagus* Species.

The males of *Achrysopophagus* are very little understood, and it does not seem that a detailed study of the males of the two Trinidad species would be justified at the present time. Professor Kirkpatrick bred series of males from unmated females of both species. Dozier (1927) described his single male of *seini* as having the general colour dark brown, and the front and mid legs pale. In Kirkpatrick's series of both the species treated here, the fronto-vertex, face and mesopleura are differing shades of metallic green. In the Trinidad material of *dactylopii* (Howard), the legs are all stramineous-white, except for the tarsi at apex, whereas in *seini* Dozier the hind femora and tibiae are in greatest part dark coloured.

Achrysopophagus sp. Domen. 1952.

Dr. G. Domenichini was able to send me two female specimens of his Peruvian species. From these, and from his excellent figures (p. 23) and description, it is clear that the species differs from both the above in its much longer head and almost entirely black sixth funicle segment. From *dactylopii* (How.) it differs also in having a strong vertical dark stripe on the cheek, and from *seini* Doz. in having the antennal scape scarcely dilated below, and the hyaline area in the lower half of the infumate part of the fore-wing scarcely extending beyond the radius and pterostigma.

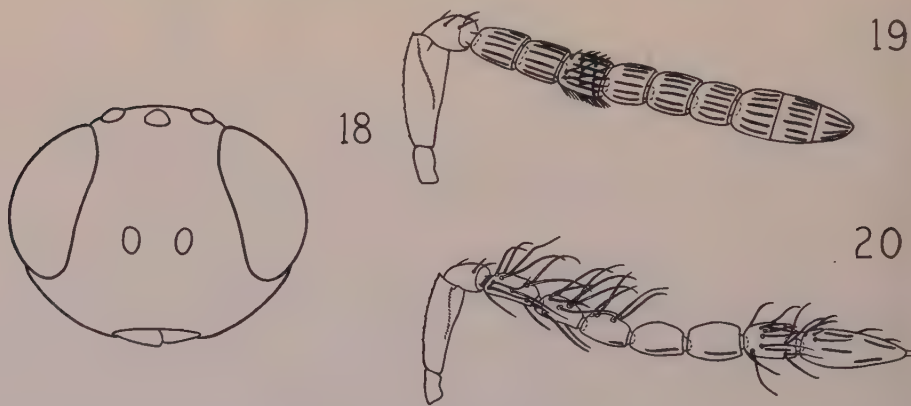
Genus **Gahaniella** Timberlake 1926.

Timberlake gave an extensive description of this genus. The new species described below is of very similar general habit to the two originally included, and conforms well with the description. The sculpture of the head, mesoscutum and scutellum are characteristic: the reticulations of the fronto-vertex are very much stronger than those of the mesoscutum. The differences that I can observe are that the "pin-punctures" of the fronto-vertex, which are weak in *saissetiae* Timb., are not distinct in the new species. The new species, like *saissetiae*, has segment 6 of the female funicle not transverse. As I see them, all three species have the thorax and especially the top of the head decidedly greenish by ordinary illumination, though the green appears weaker in strong artificial light.

My observations on the two described species are based on the original descriptions, and on the following material that Dr. Compere very kindly sent me: of *G. californica* Timb.—California, San Jacinto Mts., Idyllwild, 1 ♀, 1 ♂, ex *Lecanium corni* Bouché on Manzanita, vii.1939, P. de Bach: of *G. saissetiae* Timb.—Brazil, 1 ♀, 1 ♂, ex *Saissetia oleae* (Bern.), 1934, H. Compere; Campinas, 1 ♀, ex *Coccus viridis* (Green) on orange, 17.xi.1934, H. Compere.

Gahaniella tertia, sp.n.

Antennal sockets much nearer eyes than oral margin (fig. 18). Fronto-vertex with sculpture finer than in *saissetiae* Timb.: "pin-punctures" not distinct



Figs. 18-20.—*Gahaniella tertia*, sp.n. (18) head of female in frontal view; (19) antenna of ♀ in dextro-lateral view; (20) antenna of ♂ in same view.

The hairs drawn in figs. 19 & 20 are represented accurately; but in order to avoid overloading the figures, they have been drawn only on the following segments: fig. 19, pedicellus and flagellar 3; fig. 20, pedicellus, flagellar 1-2, 3 (part) and 6, club (part). The sensoria are represented in both figures.

on fronto-vertex, and difficult to see on face, except just near malar line. Reticulations of mesoscutum much finer than in the other two species, differing least in this respect from the male of *saissetiae* Timb. Thorax decidedly more strongly hairy than in the other two species.

General coloration not notably different from that of *californica* Timb. in most respects. Gaster bronzy above, with first large tergite mainly a bright green.

Antennae of female (fig. 19) with scape more broadened to near apex, and relatively broader than in the other two species, excluding radicle about two and two-thirds length of its greatest breadth: with first funicle segment about 1.3 times length of its greatest breadth, and sixth funicle distinctly longer than broad (20:17): with club little swollen. Scape and pedicellus dark brownish, the scape broadly at base and apex and the pedicellus basally whitish: remaining segments (as seen in dry mounts) appearing pale brownish, darkened at base. Antennae of male (fig. 20) with colouring similar to that of female, and with scape and pedicellus similar: the scape is notably different from that of *saissetiae* (Timberlake, 1926, fig. 17), but the flagellum is similar, having the club entire, and funicle segments 2-4 not nearly so strongly convex above as in *californicus* (Timberlake, fig. 15).

The leg coloration of the three species is given below in comparative form:

californica ♀—coxae metallic black with coloured reflections, brownish at apex: femora in greater part similar, the colour more distinct, merging to brownish at base and apically: tibiae metallic infusate in middle, merging to brownish, but the fore tibiae only weakly infusate on outer side: trochanters brownish: fore tibiae mainly and fore tarsi brownish, as also apical segment of mid and two apical segments of hind tarsi: mid femora at apex, mid tibiae at base and apex, and mid and hind tarsi in greater part, yellowish-white.

californica ♂—differs in that mid tibiae are only weakly infusate in middle, and are very broadly yellowish-white at apex.

saissetiae ♀—coxae similar to those of *californica* ♀, but more broadly merging to brownish: femora similar to those of *californica* ♀, but the dark part paler and less extensive: tibiae similarly infusate in middle, but paler than in *californica* and less extensively so: trochanters yellowish-white: femora and tibiae with very little brownish colouring, differing from *californica* ♀ as follows: fore tibiae mainly, and fore femora at base and apex, yellowish-white; mid femora at base and apex, and mid tibiae much more broadly at apex, yellowish-white; hind tibiae obscurely yellowish-white at base and distinctly so at apex.

saissetiae ♂—legs mainly stramineous white, with only very slight infuscations on fore legs, all trochanters and mid femora: mid and hind coxae brownish: hind femora, and mid and hind tibiae and tarsi brownish-infusate to about same extent as in ♀.

tertia ♀—legs mainly stramineous-white, including fore coxae and mid femora: mid and hind coxae metallic brownish to greenish, pale at apex: hind femora similar, pale at base and apex: hind tibiae pale brownish infusate in basal half above, indistinctly pale near base: fore tarsi pale brownish: tarsi dark only at extreme apex.

tertia ♂—similar to ♀.

Material. Trinidad, St. Augustine, 27 ♀♀ (including holotype), 28 ♂♂, hyper-parasitic on *Pseudococcus citri* (Risso) through *Leptomastix dactylopii* How. (see above), i-v.1949, T. W. Kirkpatrick.

G. tertia, sp.n. can be distinguished readily from both sexes of *californica* Timb. and from females of *saissetiae* Timb. on the pale fore coxae and mid femora. From males of *saissetiae* it must be separated on other characters.

Family THYSANIDAE.

Genus **Thysanus** Walker 1840.

Thysanus coleopratus, sp.n.

A very stout, ovoid species. Head from above broadly crescentic, 2.5 times breadth of its median length, acutely margined behind; eyes nearly but not quite reaching hind margin: ocelli in a slightly obtuse triangle, sides to base 17:25 measured from their mid points; the lateral ocelli about their own length (6 on same scale) from hind margin and about twice that from eye: head in frontal view with malar space two-thirds the longer diameter of eye; with antennae at rest not far over-reaching cheek margin; with scrobal impression semi-circular and inter-scrobal prominence an equilateral triangle. Head sculpture for the most part very finely reticulate; dorsally behind lateral ocelli very finely transversely striate; with no distinct punctures ($\times 100$). Mandibles bidentate. Antennae with scape slightly dilated below, two and a half times as long as its greatest breadth; with pedicellus one and two-thirds times length of its greatest breadth; with funicle 1-segmented (with a stalk), very small; with club large and stout, about equal in length to remainder of antenna, two and one-third times as long as broad, distinctly pointed at apex.

Pronotum short, in mid line less than one-third the length of the mesoscutum. On a dry mounted specimen, the axillae usually appear not distinctly separated from the scutellum; but in a cleared balsam preparation, a line of division can be traced. Propodeum divided, the halves well separated. Dorsum of thorax very finely transversely striate-reticulate, the reticulations suggesting very fine punctures. Penis long, almost equal to length of abdomen beyond propodeum.

Fore wings with infumations extending down from submarginal and stigma and meeting below, leaving an almost hyaline area below marginal: with marginal vein much shorter than submarginal: with marginal cilia very short, scarcely longer than thickness of marginal vein.

Mid legs with femora (fig. 25) moderately dilated below, more than three times length of their greatest breadth, showing no marked sexual dimorphism; bearing a row of small hairs that are just discernible $\times 40$, as are the pectinations of the tibial spur.

Length about 1 mm.

Head, thorax and fore coxae blackish brown: gaster and legs in varying degree paler: antennae and tarsi pale testaceous: gaster, near mid line beneath, largely stramineous.

Material. Trinidad, St. Augustine, 32 ♀♀, 26 ♂♂ *ex Pseudococcus citri* (Risso), i-v.1949, T. W. Kirkpatrick. This is suspected to be a tertiary parasite, on *Gahaniella tertia*, sp.n. (p. 800). Three females and two males are partly dissected and mounted on slides: the remaining specimens, including holotype ♀ and allotype, are on card points.

T. coleopratus, sp.n. apparently resembles *magniclavus* Dozier 1933 in its robust form and large, conspicuous club; but evidently differs from it in a number of respects. In the very short marginal cilia of the fore-wing it resembles [*Xana*] *kurdjumovi* Nik. (see below). The division of the propodeum into well-separated halves is noteworthy.

B. D. Burks informs me that the unique type of *magniclavus* was not

deposited in Washington, but was retained by Dozier in his collection. From Dozier's description, *coleoptratus*, sp.n. differs as follows:

magniclavus Dozier.

Length 0.6 mm.

Thorax with narrow, pale band between wings.

Antennal scape 5 times as long as wide.

Antennal club slightly less than 3 times as long as wide, equal in width to length of pedicellus.

Fore legs light brown, with the 3 middle tarsal segments pale.

Fore wings infumate to end of stigmal vein, with longest marginal cilia about half wing breadth.

coleoptratus, sp.n.

Length about 1 mm.

Thorax without this pale band.

Antennal scape $2\frac{1}{2}$ times as long as wide.

Antennal club $2\frac{1}{3}$ times as long as wide, distinctly wider (when flattened) than length of pedicellus.

Fore legs with tibiae and tarsi pale.

Fore wings with an almost hyaline area below marginal vein, with marginal cilia scarcely longer than width of marginal vein.

The *dipterophagus* species complex.

Girault (1916) described a species, *Signiphora dipterophaga*, reared from the pupa of a dipteran collected in a tunnel of *Diatraea* in sugar-cane in Trinidad. Two species, reared in similar circumstances, were subsequently submitted to the Commonwealth Institute of Entomology. Recently, a pair of paratypes of *dipterophagus* has been received from Washington through the good offices of Dr. B. D. Burks, and proves to agree with neither of these species, which are described below.

All three species are elongate-oval in form. They are closely related, and are best separated on the structure of the mid leg of the male. The description of the species immediately following was written before the true *dipterophaga* had been received. The other species has a pale, transverse band on the thorax of the male but not of the female, so that the sexes would be placed in different groups in the monograph of Girault (1913).

Thysanus frequentior, sp.n.

Head from above crescentic, apparently quite variable in proportion, but this may be due to partial collapse in some cases, acutely margined behind: eyes nearly but not quite reaching hind margin: ocelli in a very slightly obtuse triangle, sides to base about 20: 24 measured from their mid points; the lateral ocelli less than their own length from hind margin and not much more than their own length from eye: head in frontal view with malar space five-ninths the longer diameter of eye; with antennae at rest far over-reaching cheek margin; with scrobal impression not so broad as high. Head sculpture for the most part very finely reticulate, distinctly so $\times 60$; dorsally behind lateral ocelli more transversely striate; the reticulations suggesting scattered, very fine punctures. Mandibles bidentate. Antennae of female with scape weakly rounded above and very slightly dilated below, five times length of its greatest breadth; with pedicellus three times as long as its greatest breadth; with funicle half the length of the pedicellus, clearly 3-segmented (the basal segment with a distinct stalk), with club almost four times length of its greatest breadth, clearly shorter than remainder of antenna: antennae of male with scape much more dilated below, just over three times length of its greatest breadth; with club much more pointed to apex, well over four times length of its greatest breadth.

Pronotum only moderately short, about half length of mesoscutum in mid line. Axillae not appearing distinctly separated from scutellum in dry mounts

in either sex. Propodeum V-shaped, narrowed to middle. Pronotum and mesoscutum distinctly transversely striate, with conspicuous, scattered piliferous punctures: scutellum more finely transversely striate to reticulate, with a sub-apical transverse row of piliferous punctures: metanotum finely reticulate.

Fore wings hyaline: with marginal vein much shorter than submarginal: with marginal cilia of moderate length, those at extreme apex less, but those further round more than a quarter the greatest wing breadth.

Mid legs (figs. 21-22) with femora strongly dilated below, less than three



Figs. 21-22.—*Thysanus frequentior*, sp.n.: (21) right mid leg of ♂ in hind view; (22) right mid femur of ♀ in same view.

times length of their greatest breadth in female and scarcely twice in male; bearing rows of stout hairs that are clearly discernible $\times 25$, as are the pectinations of the tibial spur. [Similar stout hairs in rows on the mid femora are figured for [*Xana*] *kurdjumovi* Nik. by Kurdjumov (1917).]

Body colour blackish brown, with dull greenish reflections: antennal scape, tarsi, and fore and hind tibiae, very much paler: male also with mid tibiae similarly paler, and with mid femora, except in apical third and near upper margin, almost stramineous.

This species is compared with *dipterophagus* (Grlt.) (see below).

Material. Venezuela, Carabobo State, San Joaquin (450 m.), 16.xii.1947, 5 ♂♂, 14 ♀♀ (including holotype ♂ and allotype), *ex pupa* *Paratheresia claripalpis* (V.d.W.) parasitizing *Diatraea busckella* var. *rosa* Heinr. in sugar cane, em. 2.i.1948, H. E. Box: Merida State, Tovar (970 m.), 12 ♂♂, 14 ♀♀ reared from same host, iii.1947, H. E. Box: Carabobo State, Tacarigua, 13 ♀♀ reared from same host in corn, xii.1947, H. E. Box: Miranda, Charallave,

2 ♂♂, 3 ♀♀ reared from same host parasitizing *Diatraea lineolata* (Walk.) in corn, i.1947, H. E. Box: Trinidad, Cedar Hill estate, 3 ♀♀, *ex* pupa *Paratheresia claripalpis* (V.d.W.), 29.x.1930, L. D. Cleare Jr. Two males and two females from the first-mentioned series are partly dissected and are mounted on slides: the remaining specimens, including holotype ♂ and allotype, are on card points.

***Thysanus zostericus*, sp.n.**

Very closely related to *frequentior*, sp.n., differing as follows. Antennal scape much more strongly rounded above in both sexes, three times length of its greatest breadth in female, two and a quarter times in male. Thorax dorsally often somewhat less distinctly transversely striate, and more reticulate. Axillae separated from scutellum by a distinct suture in male, but not in female. Mid femur of male (fig. 24) relatively broader than in *frequentior* and with the area of spines less extensive; but that of female showing no marked species difference. Antennal club of female paler. Male with a broad, pale transverse band on the scutellum. Mid tibia of male not conspicuously pale, and mid femur not in greater part pale, but with only a conspicuous pale streak in lower half.

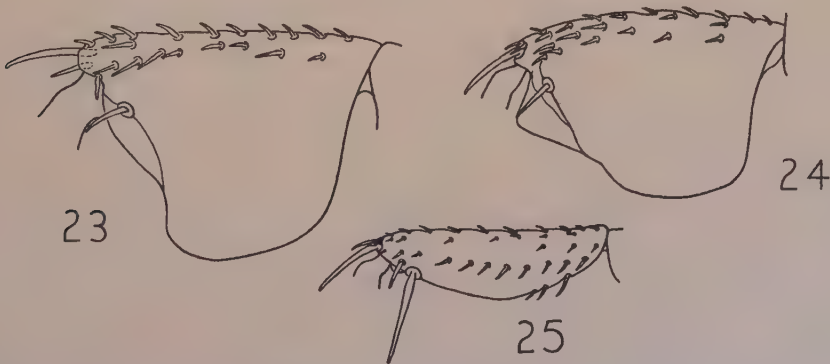
Material. Brazil, Amazonas, Santarem, 6 ♂♂, 3 ♀♀ (ref. 3601), including holotype ♂, ix.1933, 1 ♂, 3 ♀♀ (ref. 3637), ix.1933, 1 ♂, 5 ♀♀ (ref. 3710), ix.1933, 5 ♂♂, 1 ♀ (ref. 3839), x.1933, *ex* pupae of *Metagonistylum minense* Townsend (the "Amazon Fly"), J. G. Myers; Parana do Valle, 1 ♂, 5 ♀♀ (ref. 3989), 17 ix.1933, *ex* same host, J. G. Myers; Santarem, 6 ♀♀ (ref. 3630), ix.1933, *ex* pupa of *Stomatodexia diadema* (Wied.), J. G. Myers; unlocalized, 1 ♂, 13 ♀♀, ix-x.1933, *ex* Tachinid pupa, L. D. Cleare Jr.: British Guiana, N.W. district, Mabaruma, 10 ♀♀, ix.1931, *ex* Tachinid parasite of *Castnia licoides* Bdv., J. G. Myers.

***Thysanus dipterophagus* (Grlt.).**

Signiphora dipterophaga Girault, 1916, Ent. News, 27, p. 401.

Material. Trinidad, 1 ♂, 1 ♀ (paratypes).

Closely related to *frequentior*, sp.n., differing as follows. Fronto-vertex not distinctly reticulate ($\times 100$) but very finely alutaceous on a more shining background, and with distinct punctures. Antennal scape more strongly rounded above, three and a half times length of its greatest breadth in female, three times in male. Axillae separated from scutellum by a distinct furrow in male, but not



Figs. 23-25.—Right mid femur, in hind view: (23) *Thysanus dipterophagus* (Grlt.) ♂; (24) *T. zostericus*, sp.n. ♂; (25) *T. coleoptratus* sp.n. ♀.

in female. Mid femur of male (fig. 23) so strongly dilated below as to be little longer than broad (9:8), with the area of spines less extensive, and not conspicuously or in greater part pale: that of female showing no marked species difference. Female pedicellus and funicle paler: male club somewhat paler: mid tibia of male not pale.

Some of these differences it shares with *zostericus* sp.n.; but it differs from both in the head sculpture. The three species are easily separated on the form of the mid femur of the male.

It is curious that Girault did not describe the antenna; but he may have considered it as normal, and have accepted Howard's characterization of *Signiphora* as having a 3-segmented funicle.

***Thysanus elongatus* (Girault).**

Domenichini (1952) did not describe or figure the mandibles of this species; but from a card-pointed specimen and some microscopical preparations he very kindly sent me on loan, I have observed that they are bidentate. The head is of ordinary shape, not crescentic as in *dipterophagus* (Grlt.), etc. The pronotum is about half length of the mesoscutum in mid line. The axillae are not distinctly separated from the scutellum.

***Thysanus rusti* Timberlake (= *Neosigniphora nigra* Rust non *Signiphora nigra* Ashm.).**

In response to my questionnaire, B. D. Burks very kindly supplied the following information from observation on the type series. The pronotum is about one-third length of the mesoscutum in mid line. The axillae appear not distinctly separated from the scutellum. The hind wings are almost parallel-sided. The marginal cilia of the fore wings are about four-fifths length of the wing breadth.

CLASSIFICATION OF THE THYSANIDAE.

The genus *Signiphora* Ashmead, as then understood, was placed by Howard and by Ashmead in a subfamily of its own in the ENCYRTIDAE, equivalent in status to the EUPELMINAE and ENCYRTINAE. Schmiedeknecht, who classed the whole of the Chalcids as a single family, kept the Eupelmids and Encyrtids as separate subfamilies, but gave the Signiphorids the status of a tribe within the ENCYRTINAE.

Mercet (1917) and Silvestri (1918), apparently quite independently, brought together *Signiphora* Ashmead and *Thysanus* Walker, a genus that till then had been placed with the Aphelinids. Mercet treated them as separate genera within a single tribe; but Silvestri considered that *S.* (subgenus *Matritia*) *conjugal* Mercet 1916 should be placed in *Thysanus*, and probably also all the species placed by Girault (1913) in the group of *S. nigra* Ashm. A. B. Gahan has treated *Signiphora* as a straight synonym of *Thysanus*, and this is reflected in the economic entomological literature of the New World.

So far as the group category is concerned, I agree with Peck (1951), who has placed it as a separate family, equivalent in status to the EUPELMIDAE and ENCYRTIDAE. The scutellum, together with the axillae, forms a parallel-sided, strongly transverse band; the metanotum is triangular, with a differentiated, transverse basal part; the propodeum is narrow and V-shaped, or actually divided; there is no wasp-waist; and the marginal vein is not at all short. In all these characters it is very unlike the ENCYRTIDAE proper. The antennae also are unlike those of a normal Encyrtid.

The question of the generic classification interested me several years ago, for I was reluctant to accept the species then queried as *Signiphora dipterophaga*

Characters of species of Thysanidae

0—indecisive

VS—very short

Characters of species of Thysanidae		0—indecisive VS—very short		VS	
Body slender and in part parallel-sided more rotund	+	+	+	+	+
Head of normal shape	+	+	+	+	+
Head of normal shape	+	+	+	+	+
Pronotum length almost equal to mesoscutum dorsally dorsally very short	+	+	+	+	+
Antennal club (♀) longer than remainder of antenna shorter	+	0	+	+	+
Funicle of ♀ 4-segmented 3-segmented	+	+	+	+	+
Mandible 3-dentate 2-dentate	+	+	+	+	+
Marginal of fore-wings much shorter than sub-marginal sub-equal to sub-marginal	+	+	+	+	+
Spur of mid tibia much shorter than metatarsus comparable in length with metatarsus	+	+	0	+	+
Hind wings almost parallel-sided	+	+	+	+	+
not at all	+	+	+	+	+
Axillae distinctly separated	+	0	+	+	+
not distinctly separated	+	+	+	+	+
Marginal cilia of fore-wings about $\frac{1}{4}$ to $\frac{1}{2}$ wing breadth.. longer than wing breadth	+	0	+	+	+
much less than $\frac{1}{4}$ wing breadth	+	0	+	+	+

Girault as congeneric with *Thysanus ater* Walk. I made a table of apparent generic characters to separate the two but, on examining a specimen of *flavopalliata* Ashm. kindly lent me by A. B. Gahan, found it to be in some respects like *ater* and in some like the supposed *dipterophaga*.

Mercet (1916), treating of *Signiphora flavopalliata* Ashm. and two Spanish species, placed all three in separate subgenera, and stated that the characters by which he separated them were more to be considered as generic than as specific characters. In his subsequent paper (1917) he kept *Thysanus* and *Signiphora* as separate genera, and maintained his division of *Signiphora* into three subgenera.

A recent contribution to the problem is that of Nikol'skaya (1950), who put up an apparently good case for placing four Russian species all in separate genera. Her placing of *Matritia* Merc. in synonymy with *Xana* Kurdj. was apparently new, but it appears that *Matritia* was actually the earlier name. She placed *Signiphorella* Merc. in strict synonymy with *Signiphora*, on the ground that Mercet himself had grouped *flavopalliata* Ashm. with *Signiphorella merceti* Malen. It is not so clear to me why she considered, from the description of *Signiphora nigra* Ashm., that it belonged to *Xana* Kurdj.: the marginal cilia of the forewing are described by Girault (1913) as about a quarter the wing breadth, short by comparison with those of *flavopalliata* Ashm. in which they are very long, but not of comparable shortness to those figured for *kurdjumovi* Nik. (= *Xana nigra* Kurdj. non *Signiphora nigra* Ashm.). No reference was made to the paper of Compere & Smith (1928), who describe the apical marginal cilia as two-fifths the greatest wing breadth in specimens determined by A. B. Gahan [the description and figures of these authors contra-indicate Silvestri's suggested synonymy with *T. ater* Walk]. Incidentally, she placed *kurdjumovi* in her key as having the pronotum shorter than the mesoscutum, though Kurdjumov had described it as longer. She placed *elongata* Girault, together with a new species, into her new genus *Signiphorina*, but did not make it clear, from her key to the Russian species, which characters she regarded as generic and which as purely specific.

In order to seek a basis for the grouping of the Thysanid species into genera, I have tabulated characters that have been used by other authors, and those I have sought to use myself, as they occur in eleven species on which I have sufficient data, including the new species described above. *Thysanus ater* Walk. and *Neosigniphora nigra* Rust seem to be closely related; and they might be grouped together as a genus on the basis of the important mandibular character supported by the combination of others: *elongata* Girault, though described in *Neosigniphora*, would of course be excluded. In that case, the bidentate species would take the generic name *Signiphora*, since the two Foersterian names *Triphasius* and *Plastocharis* are isogenotypic with *Thysanus*. However, since in the bidentate species the other characters occur in so many different combinations, I have decided at the present time to treat all the species as falling within the genus *Thysanus* in the broad sense.

Summary.

The taxonomy of a complex of parasites associated with mealy-bugs on cacao in Trinidad is dealt with. The descriptions of nine species new to science are given and species not new have been treated in comparable detail. Fresh taxonomic or other data have been given for some related species.

The inclusion of an aberrant Thysanid species of biological importance necessitated a comparative study of characters that have been used within this group. In this connection, Thysanid parasites of TACHINIDAE attacking moth larvae in sugar-cane were considered and found separable into three closely related species, two of which are described as new.

Acknowledgements.

I wish to express my especial gratitude to Dr. H. Compere, who confirmed my placing of species within the genera *Leptomastix*, *Apoanagyrus*, *Neodiscodes* and *Aenasius*, determined the *Gahaniella* generically and as a new species, and most kindly sent me related species for study. Dr. B. D. Burks very kindly rendered the help acknowledged in the text under *Achrysopophagus* and *Thysanus*. Professor G. Ceballos made a search for material of *Thysanus* (*Matritia*) *simillima* (Merc.), but was unable to find it in the Madrid museum. Dr. G. Domenichini very kindly sent me some of his own research material, as acknowledged under *Aenasius*, *Achrysopophagus* and *Thysanus*.

For a translation of the paper by Nikol'skaya (1950) I am indebted to Madame Baribalov, of the Commonwealth Institute of Entomology.

References.

- COMPERE, H. (1931). New Encyrtid (Hymenopterous) parasites of a *Pseudococcus* species from Eritrea.—Univ. Calif. Publ. Ent., **5**, pp. 265–274.
- COMPERE, H. (1937). The species of *Aenasius*, Encyrtid parasites of mealybugs.—Proc. Hawaii. ent. Soc., **9**, pp. 383–404.
- COMPERE, H. (1938). A report on some miscellaneous African Encyrtidae in the British Museum.—Bull. ent. Res., **29**, pp. 315–337.
- COMPERE, H. (1939a). A second report on some miscellaneous African Encyrtidae in the British Museum. *Ibid.*, **30**, pp. 1–26.
- COMPERE, H. (1939b). Mealybugs and their insect enemies in South America.—Univ. Calif. Publ. Ent., **7**, pp. 57–74.
- COMPERE, H. (1947). A report on a collection of Encyrtidae with descriptions of new genera and species.—*Ibid.*, **8**, pp. 1–23.
- DOMENICHINI, G. (1951). Parassiti e iperparassiti di *Pseudococcus citri* Risso in Italia e nel Perù.—Boll. Zool. agr. Bachic., **17**, pp. 157–180.
- DOZIER, H. L. (1927). Notes on Porto Rican scale parasites.—J. Dep. Agric. P.R., **10**, pp. 267–277.
- DOZIER, H. L. (1933). Miscellaneous notes and descriptions of Chalcidoid parasites (Hymenoptera).—Proc. ent. Soc. Wash., **35**, pp. 85–100.
- GIRAULT, A. A. (1913). A systematic monograph of the Chalcidoid Hymenoptera of the subfamily Signiphorinae.—Proc. U.S. nat. Mus., **45**, pp. 189–233.
- GIRAULT, A. A. (1916). Descriptiones Hymenopterorum Chalcidoidicorum variorum cum observationibus. II.—Ent. News, **27**, pp. 401–405.
- KURDJUMOV, N. V. (1917). A new genus and species of Aphelininae (Chalcidodea).—J. appl. Ent., **1** (1916), pp. 80–81.
- MERCET, R. G. (1916). Signiforinos de España (Himenópteros Calcídidos).—Bol. Soc. esp. Hist. nat., **16**, pp. 519–533.
- MERCET, R. G. (1917). Revisión de los Signiforinos de España.—Rev. Acad. Madr., **16**, pp. 160–170.
- NIKOL'SKAYA, M. N. (1950). Representatives of the family Signiphoridae (Hymenoptera, Chalcidoidea) in the fauna of the U.S.S.R. [*In Russian*].—Dokl. Akad. Nauk S.S.S.R. (N.S.), **75**, pp. 319–321, 14 figs.
- PECK, O. (1951). Superfamily Chalcidoidea. In MUESEBECK, C. F. W. & others. Hymenoptera of America north of Mexico: synoptic catalog.—Agric. Monogr. U.S. Dep. Agric., no. 2, pp. 410–594.

SILVESTRI, F. (1918). Il genere *Thysanus* Walker (Hymenoptera: Chalcididae).
—Boll. Lab. Zool. Portici, **12**, pp. 266-271.

SMITH, H. S. & COMPERE, H. (1928). A preliminary report on the insect parasites of the Black Scale, *Saissetia oleae* (Bern.).—Univ. Calif. Publ. Ent., **4**, pp. 231-334.

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TIMBERLAKE, P. H. (1926). Miscellaneous new Chalcid-flies of the Hymenopterous family Encyrtidae.—Proc. U.S. nat. Mus., **63**, pp. 1-34.

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